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(54) Title: ANALOGS OF KERATINOCYTE GROWTH FACTOR

(57) Abstract

The present invention concerns polypeptide analogs of a potent mitogen of non-fibroblast epithelial cell growth, keratinocyte growth factor (KGF) having up to the first 24 N-terminal amino acids modified wherein cysteine residues corresponding to amino acid positions 1 and 15 of the KGF [amino acid positions 32 and 46 of SEQ ID No:2, (Cys^1 and Cys^{15}, respectively)] are deleted or substituted with another amino acid. Also disclosed are nucleic acid molecules encoding such analogs, as well as methods for using such analogs to stimulate non-fibroblast epithelial cell proliferation.

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ANALOGS OF KERATINOCYTE GROWTH FACTOR

Field of the Invention

5   The present invention relates to recombinant
DNA technology and protein engineering. Specifically,
recombinant DNA methodologies have been applied to
generate polypeptide analogs of keratinocyte growth
factor (KGF), a potent mitogen of non-fibroblast
10   epithelial cell growth, wherein the analogs have
improved stability as compared to that of the parent
KGF.

Background of the Invention

15

      The complex process of tissue generation and
regeneration is mediated by a number of protein factors
sometimes referred to as soft tissue growth factors.
These molecules are generally released by one cell type
20   and act to influence proliferation of other cell types.
(Rubin et al. (1989), Proc. Nat'l. Acad. Sci. USA,
86:802-806). Some soft tissue growth factors are
secreted by particular cell types and influence the
proliferation, differentiation, and/or maturation of
25   responsive cells in the development of multicellular
organisms (Finch et al. (1989), Science, 245:752-755).
In addition to their roles in developing organisms, some
are significant in the continued health and maintenance
of more mature systems. For instance, in mammals, there
30   are many systems where rapid cell turnover occurs. Such
systems include the skin and the gastrointestinal tract,
both of which are comprised of epithelial cells.
Included within this group of soft tissue growth facto:
is a protein family of fibroblast growth factors (FGFs).
35   There are currently eight known FGF family
members which share a relatedness among primary

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structures: basic fibroblast growth factor, bFGF
(Abraham et al. (1986), EMBO J., 5:2523-2528); acidic
fibroblast growth factor, aFGF (Jaye et al. (1986),
Science, 233:541-545); int-2 gene product, int-2
5   (Dickson & Peters (1987), Nature, 326:833); hst/kFGF
(Delli-Bovi et al. (1987), Cell, 50:729-737) and Yoshida
et al. (1987), Proc. Natl. Acad. Sci. USA, 84:7305-
7309); FGF-5 (Zhan et al. (1988), Mol. Cell. Biol.,
8:3487-3495); FGF-6 (Marics et al. (1989), Oncogene,
10   4:335-340); keratinocyte growth factor (Finch et al.
(1989), Science, 24:752-755); and hisactophilin
(Habazzettl et al. (1992), Nature, 359:855-858).

Among the FGF family of proteins, keratinocyte
growth factor ("KGF") is a unique effector of non-
15   fibroblast epithelial (particularly keratinocyte) cell
proliferation derived from mesenchymal tissues. The
term "native KGF" refers to a natural human (hKGF) or
recombinant (rKGF) polypeptide (with or without a signal
sequence) as depicted by the amino acid sequence·
20   presented in SEQ ID NO:2 or an allelic variant thereof.
[Unless otherwise indicated, amino acid numbering for
molecules described herein shall correspond to that
presented for the mature form of the native molecule
(i.e., minus the signal sequence), as depicted by amino
25   acids 32 to 194 of SEQ ID NO:2.]

Native KGF may be isolated from natural human
sources (hKGF) or produced by recombinant DNA techniques
(rKGF) (Finch et al. (1989), supra; Rubin et al. (1989),
supra; Ron et al. (1993), The Journal of Biological
30   Chemistry, 268(4):2984-2988; and Yan et al. (1991), In
Vitro Cell. Dev. Biol., 27A:437-438).

It is known that native KGF is relatively
unstable in the aqueous state and that it undergoes
chemical and physical degradation resulting in a loss
35   of biological activity during processing and storage
(Chen et al.(1994), Pharmaceutical Research, 11:1582-

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1589). Native KGF is prone also to aggregation at
elevated temperatures and it becomes inactivated under
acidic conditions (Rubin *et al.* (1989), *Proc. Natl.
Acad. Sci. USA*, **86**:802-806). Aggregation of native KGF
5   in aqueous solution also results in inactivated protein.
This is disadvantageous because such loss of activity
makes it impractical to store aqueous formulations of
native KGF proteins for extended periods of time or to
administer the protein over extended periods. Moreover,
10   this is particularly problematic when preparing
pharmaceutical formulations, because aggregated proteins
have been known to be immunogenic (Cleland *et al.*
(1993), *Crit. Rev. Therapeutic Drug Carrier Systems*,
10:307-377; Robbins *et al.* (1987), *Diabetes*, **36**:838-845;
15   and Pinckard *et al.* (1967), *Clin. Exp. Immunol.*, **2**:331-
340).

Native KGF comprises five cysteine residues,
namely amino acids 1, 15, 40, 102, and 106 (Finch *et al.*
(1989), *Science*, **24**:752-755). Although the cysteine
20   content of native KGF was reported, what has not been
reported are the roles played by cysteine residues with
respect to activity (e.g., essentiality to biological
activity) and tertiary structure (e.g., proclivity to
form undesirable intermolecular or intramolecular
25   disulfide bonds). Thus, there is no prior art teaching
of which, if any, cysteine residues are essential or are
involved in undesirable disulfide bond formation, making
the protein susceptible to aggregation and/or
instability.

30   In order to attempt to improve or otherwise
alter one or more of the characteristics of native KGF,
protein engineering may be employed. Recombinant DNA
technology has been utilized to modify the sequences of
native KGF.

35   Ron *et al.* (1993), *J. Biol. Chem.*,
268(4):2984-2988 reported modified KGF polypeptides

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having 3, 8, 27, 38 or 49 amino acids deleted from the
N-terminus. Those polypeptides missing 3, 8, or 27
N-terminal residues retained heparin binding ability;
the others did not. Also, the polypeptides missing
5 3 and 8 residues were reported as being fully active,
whereas the form missing 27 residues was 10-20 fold less
mitogenic, and the forms lacking 38 or 49 amino acids
did not have mitogenic activity. The stability of the
modified KGF polypeptides was not discussed or otherwise
10 reported.

       Published PCT application no. 90/08771, supra,
also reported the production of a chimeric protein
wherein about the first 40 N-terminal amino acids of the
mature form of native KGF were combined with the C-
15 terminal portion (about 140 amino acids) of aFGF. The
chimera was reported to target keratinocytes like KGF,
but it lacked susceptibility to heparin, a
characteristic of aFGF but not KGF. The stability of
the chimera was not discussed or otherwise reported.
20   Thus, the literature has not reported a
modified KGF molecule having significantly improved
stability relative to native KGF. Moreover, the
literature has not reported sufficient teachings or
evidence to provide a reasonable expectation of
25 successfully generating KGF molecules with such
desirable characteristics.
       Generally, the effects upon biological
activity of any amino acid change upon a protein will
vary depending upon a number of factors, including the
30 three-dimensional structure of the protein and whether
or not the modifications are to the receptor binding
region of the primary sequence of the protein. As
neither the three-dimensional structure nor the receptor
binding region on the primary structure of native KGF
35 has been published, the knowledge within the art does
not permit generalization about the effects of amino

acid modifications to native KGF based upon the effects of amino acid modifications on even commonly categorized proteins.

5   It is the object of this invention to provide polypeptide molecules encoding such analogs that exhibit enhanced stability (e.g., when subjected to typical pH, thermal and/or other storage conditions) as compared to native KGF.

Summary of the Invention

10

The present invention provides novel, biologically active polypeptide analogs of KGF. For purposes of this invention, the term "KGF" includes

15   native KGF and proteins characterized by a peptide sequence substantially the same as the peptide sequence of native KGF which retains cysteine residues corresponding to Cys^1 and Cys^{15} of native KGF (Cys^{32} and Cys^{46} of SEQ ID NO:2) and which retains some or all of

20   the biological activity of native KGF, particularly non-fibroblast epithelial cell proliferation. By "characterized by a peptide sequence substantially the same as the peptide sequence of native KGF" is meant a peptide sequence which is encoded by a DNA sequence

25   capable of hybridizing to nucleotides 201 to 684 of SEQ ID NO:1, preferably under stringent hybridization conditions.

The determination of a corresponding amino acid position between two amino acid sequences may be determined by aligning the two sequences to maximize

30   matches of residues including shifting the amino and/or carboxyl terminus, introducing gaps as required and/or deleting residues present as inserts in the candidate. Database searches, sequence analysis and manipulations

35   may be performed using one of the well-known and routinely used sequence homology/identity scanning

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algorithm programs (e.g., Pearson and Lipman (1988),
Proc. Natl. Acad. Sci. U.S.A., 85:2444-2448; Altschul et
al. (1990), *J. Mol. Biol.*, 215:403-410; Lipman and
Pearson (1985), *Science*, 222:1435 or Devereux *et al.*
5   (1984), *Nuc. Acids Res.*, 12:387-395).

           Stringent conditions, in the hybridization
context, will be stringent combined conditions of salt,
temperature, organic solvents and other parameters
typically controlled in hybridization reactions.
10 Exemplary stringent hybridization conditions are
hybridization in 4 X SSC at 62-67° C., followed by
washing in 0.1 X SSC at 62-67° C. for approximately an
hour. Alternatively, exemplary stringent hybridization
conditions are hybridization in 45-55% formamide, 4 X
15 SSC at 40-45°C. [*See*, T. Maniatis et. al., *Molecular
Cloning* (A Laboratory Manual); Cold Spring Harbor
Laboratory (1982), pages 387 to 389].

           Thus, the proteins include allelic variations,
or deletion(s), substitution(s) or insertion(s) of amino
20 acids, including fragments, chimeric or hybrid molecules
of native KGF. One example of KGF includes charge-
change polypeptides wherein one or more of amino acid
residues 41-154 of native KGF (preferably residues Arg^{41},
Gln^{43}, Lys^{55}, Lys^{95}, Lys^{128}, Asn^{137}, Gln^{138}, Lys^{139},
25 Arg^{144}, Lys^{147}, Gln^{152}, Lys^{153} or Thr^{154}) are deleted or
substituted with a neutral residue or negatively charged
residue selected to effect a protein with a reduced
positive charge (as taught in commonly owned U.S.S.N.
08/323,337, filed on October 13, 1994), specifically
30 including R(144)Q, a KGF having a substitution of
glutamine for arginine at amino acid positions 144 of
native KGF. Another example of KGF includes proteins
generated by substituting at least one amino acid having
a higher loop-forming potential for at least one amino
35 acid within a loop-forming region of Asn^{115}-His^{116}-
Tyr^{117}-Asn^{118}-Thr^{119} of native KGF (as taught in

commonly owned U.S.S.N. 08/323,473, filed on October 13, 1994), specifically including H(116)G, a KGF having a substitution of glycine for histidine at amino acid position 116 of native KGF. A still further example
5   includes proteins having one or more amino acid substitutions, deletions or additions within a region of 123-133 (amino acids 154-164 of SEQ ID NO:2) of native KGF; these proteins may have agonistic or antagonistic activity.

10   Surprisingly, it has been discovered when a KGF molecule (i.e., parent molecule) having cysteine residues corresponding (as determined using techniques described above) to Cys^1 and Cys^{15} of native KGF (cysteine residues 32 and 46 of SEQ ID NO:2) is modified
15   by replacing the corresponding cysteines, the resultant KGF analog has improved stability as compared to the parent molecule. Preferably, in addition to having increased stability, the invention is directed to those analogs which also exhibit full biological activity
20   (i.e., at least substantially similar receptor binding or affinity) as compared to native KGF.

        In another aspect of the invention, purified and isolated nucleic acid molecules encoding the various biologically active polypeptide analogs of KGF are
25   described. In one embodiment, such nucleic acids comprise DNA molecules cloned into biologically functional plasmid or viral vectors. In another embodiment, nucleic acid constructs may then be utilized to stably transform a procaryotic or eucaryotic host
30   cell. In still another embodiment, the invention involves a process wherein either a procaryotic (preferably *E. coli*) or eucaryotic host cell stably transformed with a nucleic acid molecule is grown under suitable nutrient conditions in a manner allowing the
35   expression of the KGF analog. Following expression, the

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resultant recombinant polypeptide can be isolated and purified.

A further aspect of the invention concerns pharmaceutical formulations comprising a therapeutically effective amount of a KGF analog and an acceptable pharmaceutical carrier. Such formulations will be useful in treating patients afflicted with epithelial diseases and injuries.

In this vein, another aspect relates to methods of stimulating epithelial cell growth by administering to a patient a therapeutically effective amount of a KGF analog. In one embodiment, non-fibroblast epithelial cells are the cells whose proliferation is stimulated. Such epithelial cells include various adnexal cells, pancreatic cells, liver cells, and mucosal epithelium in the respiratory and gastrointestinal tracts.

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Brief Description of the Figures

Figure 1 shows the nucleotide (SEQ ID NO:1) and amino acid (SEQ ID NO:2) sequences of native KGF
5   (the nucleotides encoding the mature form of native KGF is depicted by bases 201 to 684 of SEQ ID NO:1 and the mature form of KGF is depicted by amino acid residues 32 to 194 of SEQ ID NO:2).

Figures 2A, 2B and 2C show the plasmid maps of
10 pCFM1156, pCFM1656 and pCFM3102, respectively.

Figure 3 shows the nucleotide (SEQ ID NO:3) and amino acid (SEQ ID NO:4) sequences of the construct RSH-KGF.

Figure 4 shows the nucleotide (SEQ ID NO:5)
15 and amino acid (SEQ ID NO:6) sequences of the construct contained in plasmid KGF.

Figure 5 shows the chemically synthesized OLIGOs (OLIGO#6 through OLIGO#11; SEQ ID NO:12-17, respectively) used to substitute the DNA sequence
20 between a KpnI site and an EcoRI site (from amino acid positions 46 to 85 of SEQ ID No:6) in the construct contained in plasmid KGF to produce the construct in plasmid KGF(dsd).

Figure 6 shows the chemically synthesized
25 OLIGOs (OLIGO#12 through OLIGO#24; SEQ ID NO:18-30, respectively) used to construct KGF (codon optimized).

Figure 7 shows the nucleotide (SEQ ID NO:31) and amino acid (SEQ ID NO:32) sequences of C(1,15)S, a KGF analog having substitutions of serine for cysteine
30 at amino acid positions 1 and 15 of native KGF.

Figure 8 shows the nucleotide (SEQ ID NO:33) and amino acid (SEQ ID NO:34) sequences of ΔN3/C(15)S, a KGF analog having a deletion of the first 3 amino acids of the N-terminus and a substitution of serine for
35 cysteine at amino acid position 15 of native KGF.

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        Figure 9 shows the nucleotide (SEQ ID NO:35)
and amino acid (SEQ ID NO:36) sequences of ΔN3/C(15)-, a
KGF analog having a deletion of the first 3 amino acids
of the N-terminus and a deletion of cysteine at amino
5   acid position 15 of native KGF.

        Figure 10 shows the nucleotide (SEQ ID NO:37)
and amino acid (SEQ ID NO:38) sequences of ΔN8/C(15)S, a
KGF analog having a deletion of the first 8 amino acids
of the N-terminus and a substitution of serine for
10 cysteine at amino acid position 15 of native KGF.

        Figure 11 shows the nucleotide (SEQ ID NO:39)
and amino acid (SEQ ID NO:40) sequences of ΔN8/C(15)-, a
KGF analog having a deletion of the first 8 amino acids
of the N-terminus and a deletion of cysteine at amino
15 acid position 15 of native KGF.

        Figure 12 shows the nucleotide (SEQ ID NO:41)
and amino acid (SEQ ID NO:42) sequences of ΔN15, a KGF
analog having a deletion of the first 15 amino acids of
the N-terminus of native KGF.

20   Figure 13 shows the nucleotide (SEQ ID NO:43)
and amino acid (SEQ ID NO:44) sequences of ΔN16, a KGF
analog having a deletion of the first 16 amino acids of
the N-terminus of native KGF.

        Figure 14 shows the nucleotide (SEQ ID NO:45)
25 and amino acid (SEQ ID NO:46) sequences of ΔN17, a KGF
analog having a deletion of the first 17 amino acids of
the N-terminus of native KGF.

        Figure 15 shows the nucleotide (SEQ ID NO:47)
and amino acid (SEQ ID NO:48) sequences of ΔN18, a KGF
30 analog having a deletion of the first 18 amino acids of
the N-terminus of native KGF.

        Figure 16 shows the nucleotide (SEQ ID NO:49)
and amino acid (SEQ ID NO:50) sequences of ΔN19, a KGF
analog having a deletion of the first 19 amino acids of
35 the N-terminus of native KGF.

Figure 17 shows the nucleotide (SEQ ID NO:51) and amino acid (SEQ ID NO:52) sequences of ΔN20, a KGF analog having a deletion of the first 20 amino acids of the N-terminus of native KGF.

5   Figure 18 shows the nucleotide (SEQ ID NO:53) and amino acid (SEQ ID NO:54) sequences of ΔN21, a KGF analog having a deletion of the first 21 amino acids of the N-terminus of native KGF.

Figure 19 shows the nucleotide (SEQ ID NO:55) '
10   and amino acid (SEQ ID NO:56) sequences of ΔN22, a KGF analog having a deletion of the first 22 amino acids of the N-terminus of native KGF.

Figure 20 shows the nucleotide (SEQ ID NO:57) and amino acid (SEQ ID NO:58) sequences of ΔN23, a KGF
15   analog having a deletion of the first 23 amino acids of the N-terminus of native KGF.

Figure 21 shows the nucleotide (SEQ ID NO:59) and amino acid (SEQ ID NO:60) sequences of ΔN24, a KGF analog having a deletion of the first 24 amino acids of
20   the N-terminus of native KGF.

Figure 22 shows the nucleotide (SEQ ID NO:61) and amino acid sequences (SEQ ID NO:62) of C(1,15)S/R(144)E, a KGF analog having substitutions of serine for cysteine at amino acid positions 1 and 15 and
25   a substitution of glutamic acid for arginine at amino acid position 144 of native KGF.

Figure 23 shows the nucleotide (SEQ ID NO:63) and amino acid (SEQ ID NO:64) sequences of C(1,15)S/R(144)Q, a KGF analog having substitutions of
30   serine for cysteine at amino acid positions 1 and 15 and a substitution of glutamine for arginine at amino acid position 144 of native KGF.

Figure 24 shows the nucleotide (SEQ ID NO:65) and amino acid (SEQ ID NO:66) sequences of ΔN23/R(144)Ç
35   a KGF analog having a deletion of the first 23 amino acids of the N-terminus and a substitution of glutaminᴇ

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for arginine at amino acid position 144 of native KGF.

Figure 25 shows the amount of remaining soluble protein when native KGF and C(1,15)S were stored in 20 mM Na phosphate, 0.15 M NaCl, pH 7.0 at 37˙C for
5   27 hrs.

Figure 26 shows the amount of remaining soluble protein when native KGF and C(1,15)S were stored in 0.15 M NaCl, 20 mM NaPO$_4$, 0.15 M NaCl, pH 7.0 at 37˙C for 27 hrs.
10   Figure 27 shows the amount of remaining soluble protein when native KGF, ΔN15 and C(1,15)S were stored in 50 mM NaPO$_4$, 0.15 M NaCl, pH 7.0 at 37˙C for 27 hrs.

Figure 28 shows the amount of soluble protein,
15 determined by size exclusion HPLC, as a function of incubation time at 37°C.

Figure 29 shows the estimated melting temperature (T$_m$) as a function of pH for native KGF, C(1,15)S/R(144)Q and C(1,15)S/R(144)E.
20   Figure 30 shows a typical profile of mitogenic activity of C(1,15)S determined by measuring the incorporation of [^{3}H]-Thymidine during DNA synthesis by comparing it to a native KGF standard curve.

Figure 31 shows a typical profile of the
25 mitogenic activity of ΔN15 determined by measuring the incorporation of [^{3}H]-Thymidine during DNA synthesis and by comparing it to a native KGF standard curve.

Figure 32 shows a typical profile of the mitogenic activity of ΔN23 determined by measuring the
30 incorporation of [^{3}H]-Thymidine during DNA synthesis and by comparing it to a native KGF standard curve.

Figure 33 shows a typical profile of the mitogenic activity of ΔN23/R(144)Q determined by measuring the incorporation of [^{3}H]-Thymidine during DNA
35 synthesis and by comparing it to a native KGF standard curve.

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Figure 34 shows a typical profile of the mitogenic activity of C(1,15)S/R(144)Q determined by measuring the incorporation of [3H]-Thymidine during DNA synthesis and by comparing it to a native KGF standard curve.

Figure 35 shows a typical profile of the mitogenic activity of C(1,15)S/R(144)E determined by measuring the incorporation of [3H]-Thymidine during DNA synthesis and by comparing it to a native KGF standard curve.

Figure 36 shows the effects of C(1,15)S and ΔN23 on serum chemistries.

Figure 37 shows the nucleotide (SEQ ID NO:77) and amino acid (SEQ ID NO:78) sequences of C(1,15,40)S, a KGF analog having substitutions of serine for cysteine at amino acid positions 1, 15 and 40 of native KGF.

Figure 38 shows the nucleotide (SEQ ID NO:79) and amino acid (SEQ ID NO:80) sequences of C(1,15,102)S, a KGF analog having substitutions of serine for cysteine at amino acid positions 1, 15 and 102 of native KGF.

Figure 39 shows the nucleotide (SEQ ID NO:81) and amino acid (SEQ ID NO:82) sequences of C(1,15,102,106)S, a KGF analog having substitutions of serine for cysteine at amino acid positions 1, 15, 102 and 106 of native KGF.

Figure 40 shows the nucleotide (SEQ ID NO:83) and amino acid (SEQ ID NO:84) sequences of ΔN23/N(137)E, a KGF analog having a deletion of the first 23 amino acids of the N-terminus and a substitution of glutamic acid for asparagine at amino acid position 137 of native KGF.

Figure 41 shows the nucleotide (SEQ ID NO:85) and amino acid (SEQ ID NO:86) sequences of ΔN23/K(139)E, a KGF analog having a deletion of the first 23 amino acids of the N-terminus and a substitution of glutamic

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acid for lysine at amino acid position 139 of native
KGF.

Figure 42 shows the nucleotide (SEQ ID NO:87)
and amino acid (SEQ ID NO:88) sequences of ΔN23/K(139)Q,
5   a KGF analog having a deletion of the first 23 amino
acids of the N-terminus and a substitution of glutamine
for lysine at amino acid position 139 of native KGF.

Figure 43 shows the nucleotide (SEQ ID NO:89)
and amino acid (SEQ ID NO:90) sequences of ΔN23/R(144)A,
10   a KGF analog having a deletion of the first 23 amino
acids of the N-terminus and a substitution of alanine
for arginine at amino acid position 144 of native KGF.

Figure 44 shows the nucleotide (SEQ ID NO:91)
and amino acid (SEQ ID NO:92) sequences of ΔN23/R(144)E,
15   a KGF analog having a deletion of the first 23 amino
acids of the N-terminus and a substitution of glutamic
acid for arginine at amino acid position 144 of native
KGF.

Figure 45 shows the nucleotide (SEQ ID NO:93)
20   and amino acid (SEQ ID NO:94) sequences of ΔN23/R(144)L,
a KGF analog having a deletion of the first 23 amino
acids of the N-terminus and a substitution of leucine
for arginine at amino acid position 144 of native KGF.

Figure 46 shows the nucleotide (SEQ ID NO:95)
25   and amino acid (SEQ ID NO:96) sequences of ΔN23/K(147)E,
a KGF analog having a deletion of the first 23 amino
acids of the N-terminus and a substitution of glutamic
acid for lysine at amino acid position 147 of native
KGF.   ‐

30   Figure 47 shows the nucleotide (SEQ ID NO:97)
and amino acid (SEQ ID NO:98) sequences of ΔN23/K(147)Q,
a KGF analog having a deletion of the first 23 amino
acids of the N-terminus and a substitution of glutamine
for lysine at amino acid position 147 of native KGF.

35   Figure 48 shows the nucleotide (SEQ ID NO:99)
and amino acid (SEQ ID NO:100) sequences of

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ΔN23/K(153)E, a KGF analog having a deletion of the
first 23 amino acids of the N-terminus and a
substitution of glutamic acid for lysine at amino acid
position 153 of native KGF.

5   Figure 49 shows the nucleotide (SEQ ID NO:101)
and amino acid (SEQ ID NO:102) sequences of
ΔN23/K(153)Q, a KGF analog having a deletion of the
first 23 amino acids of the N-terminus and a
substitution of glutamine for lysine at amino acid
10   position 153 of native KGF.

          Figure 50 shows the nucleotide (SEQ ID NO:103)
and amino acid (SEQ ID NO:104) sequences of
ΔN23/Q(152)E/K(153)E, a KGF analog having a deletion of
the first 23 amino acids of the N-terminus and a
15   substitution of glutamic acid for glutamine at amino
acid position 152 of native KGF and glutamic acid for
lysine at amino acid position 153 of native KGF.

          Figure 51 shows the effect of ΔN23 on
streptozotocin-induced diabetes in Sprague-Dawley rats.
20

Detailed Description

          In accordance with the present invention,
novel analogs of KGF are provided. It has now been
25   determined that four of the cysteine residues of native
KGF (Cys^1 and Cys^{15}, and Cys^{102} and Cys^{106}) are involved
in the formation of two disulfide bridges. Cys^{40} is not
involved in intramolecular disulfide formation. Hence,
KGF contains two small disulfide loops separated by
30   almost 90 amino acids. Based upon first principals, the
determination of which cysteine residues are involved in
the disulfide bridges suggests which cysteines are free
to form undesirable intermolecular crosslinks or
intramolecular bonds that cause the protein to adopt an
35   undesirable tertiary structure (e.g., a conformation
that reduces the activity of the protein).

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Surprisingly, it has been found that modifying a KGF by deleting or substituting amino acid residues for the cysteine residues corresponding to positions 1 and 15 of KGF (positions 32 and 46 of SEQ ID NO:2)

5   produces a KGF analog having substantially improved stability (i.e., reduced problems caused by improper refolding, intermolecular disulfide formation and/or protein aggregation). For example, the KGF analogs will generally be purified in a greater yield of soluble,

10   correctly folded protein. Moreover, once the material is purified, it will be more stable to pH, temperature, etc. as compared to the stability of the parent molecule. Although not intended to be bound by theory, it is believed that Cys^1 and Cys^{15} of KGF in addition to

15   forming an intramolecular disulfide bridge and an N-terminal disulfide loop, under certain conditions also exist as free cysteines which are capable of forming intermolecular disulfide bridges, resulting in protein instability and aggregation. Moreover, it has been

20   discovered that deletion of the N-terminal disulfide loop is not important for receptor binding or mitogenic activity.

As used in this invention, a "KGF analog" or a "polypeptide analog of KGF" shall mean any of the

25   described naturally and non-naturally occurring polypeptides differing in structure from a KGF by possessing modifications in peptide sequence corresponding to the 24 amino acid N-terminus of the KGF (amino acids 32 to 55 of SEQ ID NO:2), wherein Cys^1 and

30   Cys^{15} of the KGF (amino acids 32 to 46 of SEQ ID NO:2) are replaced or deleted. The manner by which the cysteines are replaced or deleted is not significant and includes, for example, polypeptide analogs incorporating one or more amino acid deletions and/or substitutions.

35   Accordingly, the invention provides a family of novel

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keratinocyte growth factor proteins. This family
comprises several groups of proteins.

One group of KGF analogs includes molecules in
which the cysteines at positions 1 and 15 of a KGF are
5   replaced with amino acids, including those that do not
occur naturally in proteins. Strategies for generating
the substitution analogs include using site-directed
mutagenesis (Ho et al. (1989), Gene, 77:51-59; Innis
et al. "PRC Protocols", Academic Press, Inc., San Diego,
10   CA). [KGF analogs comprising an amino acid substitution
are referred to by the residue found at that position in
the mature protein (minus signal sequence) set forth in
SEQ ID NO:2, followed by that amino acid position in
parentheses and the new amino acid. For instance, an
15   analog comprising a cysteine to serine substitution at
amino acid position 15 of KGF (position 46 of SEQ ID
NO:2) is referred to as "C(15)S".] Preferably, the
cysteine is converted to a neutral amino acid such as
glycine, valine, alanine, leucine, isoleucine, tyrosine,
20   phenylalanine, histidine, tryptophan, serine, threonine
and methionine, with serine, threonine and alanine being
most preferred because of their chemical similarity to
cysteine. Example 1 details the generation of C(1,15)S
using partial gene synthesis (in conjunction with other
25   recombinant techniques) followed by recombinant
expression in a stably transformed bacterial host.

Another group of KGF analogs includes
molecules that have the cysteines at positions 1 and 15
deleted from KGF. Different strategies may be employed
30   in developing such KGF analogs, such as N-terminal
truncations and site-specific deletions, or a
combination of both.

An "N-terminal truncation" refers to a
modification of a KGF wherein 1 to 24 N-terminal amino
35   acid residues of the KGF (amino acids 32 to 55 of SEQ ID
NO:2), including Cys^1 and Cys^{15}, have been deleted.

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[KGF analogs comprising a truncation of amino acids will be referred to by the residue deleted at that position in the mature protein (minus signal sequence) set forth in SEQ ID NO:2, beginning with the site of the deletion

5   and by the number of residues deleted. For instance, a KGF analog comprising an N-terminal truncation of 24 residues of the KGF (residues 1-55 of SEQ ID NO:2) will be referred to as a "ΔN24" analog.] Specifically included within this group are ΔN23 analogs of native

10   KGF wherein one or more of amino acid residues 41-154 (amino acids 72-185 of SEQ ID NO:2), specifically including amino acid residues 123-133 (amino acids 154-164 of SEQ ID NO:2), are deleted or substituted with a neutral residue or negatively charged residue selected

15   to effect a protein with a reduced positive charge. Preferred residues for modification are Arg^{41}, Gln^{43}, Lys^{55}, Lys^{95}, Lys^{128}, Asn^{137}, Gln^{138}, Lys^{139}, Arg^{144}, Lys^{147}, Gln^{152}, Lys^{153} or Thr^{154}, with Gln^{138}, Lys^{139}, Arg^{144}, Lys^{147}, Gln^{152} or Lys^{153} being more preferred and

20   Arg^{144} being most preferred Also included within this group are ΔN23 analogs of native KGF having loop-forming modifications within a loop-forming region of Asn^{115}-His^{116}-Tyr^{117}-Asn^{118}-Thr^{119} (amino acids 146-150 of SEQ ID NO:2), preferably including a charge-change

25   modification of amino acid residue 116, more preferably the substitution of Gly at position 116. Further still, included in this group are ΔN23 analogs of native KGF having one or more amino acid substitutions, deletions or additions within a region of 123-133 (amino acids of

30   154-164 SEQ ID NO:2) of native KGF.
         Example 1 details the generation of systematic N-terminal truncations accomplished using partial gene synthesis, in conjunction with other recombinant techniques, followed by recombinant expression in a

35   stably transformed bacterial host. Such exemplified N-terminal truncations include ΔN15 to ΔN24. Moreover,

Example 3 details expression in mammalian cell culture
cells of DNA encoding native KGF and heterogeneous N-
terminal glycosylated isoforms, the purification of
preferably a glycosylated isoform having an N-terminal
5   truncation of amino acids 1-23 of the mature form of
native KGF.

In contrast, a site-specific deletion refers
to a modification of a KGF wherein one or more amino
acid residues (e.g., Cys^1 or Cys^{15}) are removed. When a
10 Cys^1 or Cys^{15} of the KGF is specifically deleted, the
analog is one amino acid shorter than the KGF. [KGF
analogs comprising an amino acid deletion are referred
to by the residue found at that position in the mature
protein (minus signal sequence) set forth in SEQ ID
15 NO:2, followed by that amino acid position in
parentheses, and a negative sign. For instance, an
analog within this group comprising a deletion of
cysteine at position 15 of the KGF (position 36 of SEQ
ID NO:2) is referred to as "C(15)-".] The site-specific
20 deletions may be generated using site directed
mutagenesis, as described above.

Also included within this group are analogs in
which Cys^1 and Cys^{15} of a KGF are removed through
truncation and deletion. For example, representative
25 KGF analogs comprise the truncation of the first three
amino terminal residues (Cys-Asn-Asp) of the KGF or the
truncation of the first eight amino acids (Cys-Asn-Asp-
Met-Thr-Pro-Glu-Gln) of the KGF coupled with the
deletion of cysteine at amino acid position 15 of KGF
30 (these analogs are referred to as ΔN3/C(15)- and
ΔN8/C(15)-, respectively). As a methionine residue
occurs naturally at the fourth and ninth amino acid
position in native KGF, no additional Met codon is
required to enable proper initiation of translation.
35 A still further group includes molecules in
which the cysteines at positions 1 and 15 of KGF are

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replaced through truncation and substitution. For
example, representative KGF analogs are ΔN3/C(15)S, and
ΔN8/C(15)S. Such analogs comprise the truncation of the
first three amino-terminal residues of a KGF or the
5   deletion of the first eight amino-terminal residues of a
KGF coupled with the substitution of cysteine at amino
acid position 15 with another amino acid, for instance,
serine.
When the KGF analogs are biologically
10   generated, i.e., are the products of cellular expression
as opposed to the products of solid state synthesis,
proteolytic or enzymatic derivatization of naturally-
occurring products, etc., the nucleic acids encoding
such polypeptides, will differ in one or more
15   nucleotides as compared to the native KGF nucleotide
sequence. Such nucleotides may be expressed and the
resultant polypeptide purified by any one of a number of
recombinant technology methods known to those skilled in
the art.
20   DNA sequences coding for all or part of the
KGF analogs may include among other things the
incorporation of codons "preferred" for expression in
selected host cells (e.g., "E. coli expression codons");
the provision of sites for cleavage by restriction
25   enzymes; and the provision of additional initial,
terminal, or intermediate nucleotide sequences (e.g., an
initial methionine amino acid residue for expression in
E. coli cells), to facilitate construction of readily
expressed vectors.
30   The present invention also provides
recombinant molecules or vectors for use in the method
of expression of the polypeptides. Such vectors may be
comprised of DNA or RNA and can be circular, linear,
single-stranded or double-stranded in nature and can be
35   naturally-occurring or assemblages of a variety of
components, be they naturally-occurring or synthetic.

Many examples of such expression vectors are known. The components of the vectors, e.g. replicons, selection genes, enhancers, promoters, and the like, may be obtained from natural sources or synthesized by known procedures. In each case, expression vectors useful in this invention will contain at least one expression control element functionally associated with the inserted nucleic acid molecule encoding the KGF polypeptide analog. This control element is responsible for regulating polypeptide expression from the nucleic acid molecules of the invention. Useful control elements include, for example, the *lac* system, the *trp* system, the operators and promoters from phage λ, a glycolytic yeast promoter, a promoter from the yeast acid phosphatase gene, a yeast alpha-mating factor, and promoters derived from adenovirus, Epstein-Barr virus, polyoma, and simian virus as well as those from various retroviruses. However, numerous other vectors and control elements suitable for procaryotic or eucaryotic expression are known in the art and may be employed in the practice of this invention.

Examples of suitable procaryotic cloning vectors may include plasmids from *E. coli* (e.g. pBR322, col E1, pUC, and the F-factor), with preferred plasmids being pCFM1156 (ATCC 69702), pCFM1656 (ATCC 69576) and pCFM3102 (described in the Examples section, below). Other appropriate expression vectors of which numerous types are known in the art for mammalian, insect, yeast, fungal and bacterial expression can also be used for this purpose. The transfection of these vectors into appropriate host cells can result in expression of the KGF analog polypeptides.

Host microorganisms useful in this invention may be either procaryotic or eucaryotic. Suitable procaryotic hosts include various *E. coli* (e.g., FM5, HB101, DH5α, DH10, and MC1061), *Pseudomonas*, *Bacillus*,

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and *Streptomyces* strains, with *E. coli* being preferred.
Suitable eucaryotic host cells include yeast and other
fungi, insect cells, plant cells and animal cells, such
as COS (e.g., COS-1 and COS-7) and CV-1 monkey cell
5   lines, 3T3 lines derived from Swiss, Balb-c or NIH
cells, HeLa and L-929 mouse cells, and CHO, BHK or HaK
hamster cells. Depending upon the host employed,
recombinant polypeptides produced in accordance herewith
will be glycosylated with mammalian or other eucaryotic
10 carbohydrates or may be non-glycosylated.
        The preferred production method will vary
depending upon many factors and considerations; the
optimum production procedure for a given situation will
be apparent to those skilled in the art through minimal
15 experimentation. The resulting expression product may
then be purified to near homogeneity using procedures
known in the art. A typical purification procedure for
procaryotic cell production involves rupturing the cell
walls by high pressure or other means, centrifugation or
20 filtration to remove cellular debris followed by ion
exchange chromatography of supernatant or filtrate and,
finally, hydrophobic interaction chromatography. If the
analog is expressed in insoluble form, another
purification technique involves first solublizing the
25 inclusion bodies containing the analogs followed by ion
exchange chromatography, then refolding of the protein,
and, finally, hydrophobic interaction chromatography.
Exemplary purification techniques are taught in commonly
owned U.S.S.N. 08/323,339, filed on October 13, 1994.
30 Generally, U.S.S.N. 08/323,339 teaches a method for
purifying a keratinocyte growth factor comprising: (a)
obtaining a solution comprising the KGF; (b) binding the
KGF from the solution of part (a) to a cation exchange
resin; (c) eluting the KGF in an eluate solution from
35 the cation exchange resin; (d) either passing the eluate
solution from part (c) through an appropriate molecular

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weight exclusion matrix or performing hydrophobic
interaction chromatography on the eluate solution of
part (c); and (e) recovering the KGF from the molecular
weight exclusion matrix or hydrophobic interaction
5   chromatography.

         Of course, the analogs may be rapidly screened
to assess their physical properties. The Examples
section set forth various well-known stability assays,
although the specific assay used to test the analog is
10   not critical. Moreover, the level of biological
activity (e.g., receptor binding and/or affinity,
mitogenic, cell proliferative and/or in vivo activity)
may also be tested using a variety of assays, some of
which are set forth in the Examples section. Numerous
15   assays are well-known and can be used to quickly screen
the KGF analogs to determine whether or not they possess
acceptable biological activity. One such assay
specifically tests the KGF analogs for the ability to
bind to the KGF receptor (KGFR) by competing with
20   ^{125}I-KGF binding (Bottaro et al. (1990), *J. Biol. Chem.*,
265:12767-12770; Ron et al. (1993), *J. Biol. Chem.*,
268:2984-2988). An alternative method for assaying
KGFR/KGF analog interactions involves the use of
techniques such as real time biospecific interaction
25   analysis (BIA) (Felder et al. (1993), *Molecular &
Cellular Biology*, **13**:1449-1455). Additionally a
mitogenic assay can be utilized to test the ability of
the KGF analogs to stimulate DNA synthesis (Rubin et al.
(1989), *supra*). Finally, cell proliferative assays can
30   be utilized to test the ability of the KGF analogs to
stimulate cell proliferation (Falco, et al. (1988),
Oncogene, **2**:573-578). Using any of the aforementioned
assay systems, KGF analogs can be rapidly screened for
their biological activity.
35   In a preferred embodiment, the present
invention is directed to KGF analogs which retain the

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full (i.e., at least substantially similar) *in vitro*
or *in vivo* biological activity to that of native KGF.
Exemplary KGF analogs with these properties, as
determined by one or more of the above assays,

5   are C(1,15)S, ΔN3/C(15)S, ΔN3/C(15)-, ΔN8/C(15)S,
ΔN8/C(15)-, ΔN15, ΔN16, ΔN17, ΔN18, ΔN19, ΔN20, ΔN21,
ΔN22, ΔN23, ΔN24 or ΔN23/R(144)Q.

The KGF analogs may be further modified to
contain additional chemical moieties not normally a part

10 of the peptide. Such derivatized moieties may improve
the solubility, absorption, biological half life, and
the like of the KGF analog. The moieties may
alternatively eliminate or attenuate any undesirable
side effects of the protein and the like. Moieties

15 capable of mediating such effects are disclosed, for
example, in *REMINGTON'S PHARMACEUTICAL SCIENCES*, 18th
ed., Mack Publishing Co., Easton, PA (1990). Covalent
modifications may be introduced into the molecule by
reacting targeted amino acid residues of the peptide

20 with an organic derivatizing agent that is capable of
reacting with selected side chains or terminal residues
(T.E. Creighton (1983), *PROTEINS: STRUCTURE AND MOLECULE
PROPERTIES*, W.H. Freeman & Co., San Francisco,
pp. 79-86). Polyethylene glycol ("PEG") is one such

25 chemical moiety which has been used in the preparation
of therapeutic protein products. For some proteins, the
attachment of polyethylene glycol has been shown to
protect against proteolysis, Sada, et al.(1991), *J.
Fermentation Bioengineering*, 71:137-139 , and methods

30 for attachment of certain polyethylene glycol moieties
are available. See U.S. Patent No. 4,179,337, Davis et
al., "Non-Immunogenic Polypeptides," issued December 18,
1979; and U.S. Patent No. 4,002,531, Royer, "Modifying
enzymes with Polyethylene Glycol and Product Produced

35 Thereby," issued January 11, 1977. For a review, see
Abuchowski et al., in Enzymes as Drugs. (Holcerberg and

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Roberts, (eds.) pp. 367-383 (1981)). For polyethylene
glycol, a variety of means have been used to attach the
polyethylene glycol molecules to the protein.
Generally, polyethylene glycol molecules are connected

5   to the protein via a reactive group found on the
protein. Amino groups, such as those on lysine residues
or at the N-terminus, are convenient for such
attachment. For example, Royer (U.S. Pat.
No. 4,002,531, above) states that reductive alkylation

10   was used for attachment of polyethylene glycol molecules
to an enzyme. EP 0 539 167, published April 28, 1993,
Wright, "Peg Imidates and Protein Derivates Thereof"
states that peptides and organic compounds with free
amino group(s) are modified with an imidate derivative

15   of PEG or related water-soluble organic polymers. U.S.
Patent No. 4,904,584, Shaw, issued February 27, 1990,
relates to the modification of the number of lysine
residues in proteins for the attachment of polyethylene
glycol molecules via reactive amine groups.

20   In yet another embodiment, the present
invention is directed to a single-dose administration
unit of a medicinal formulation, which can be safely
administered parenterally or orally to treat a disease
in a warm-blooded animal (such as a human). Such

25   medicinal formulation may be in the form of a
lyophilized or otherwise dehydrated therapeutic or
diagnostic which can be reconstituted by the addition of
a physiologically acceptable solvent. The solvent may
be any media such as sterile water, physiological saline

30   solution, glucose solution or other aqueous
carbohydrates (e.g., polyols such as mannitol, xylitol
or glycerol) which is capable of dissolving the dried
composition, which is compatible with the selected
administration route and which does not negatively

35   interfere with the active principle and the
reconstitution stabilizers employed. In a specific

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embodiment, the present invention is directed to a kit
for producing the single-dose administration unit. The
kit contains both a first container having a dried
protein and a second container having an aqueous
5   formulation comprising a reconstitution stabilizer. As
for the concentration of the protein in the solution,
the solution volume which is charged into each
container, and the capacity of the containers
(interrelated parameters which can be suitably modified,
10   depending upon the desired concentration of active
principle in the end-dosage unit), these may vary within
wide ranges well-known to skilled artisans.

KGF analogs according to the invention may be
useful as therapeutic and diagnostic agents and as
15   research reagents. Thus the KGF analogs may be used in
in vitro and/or in vivo diagnostic assays to quantify
the amount of KGF in a tissue or organ sample or to
determine and/or isolate cells which express KGFR
(Bottaro et al. (1990), J. Biol. Chem., 265:12767-12770;
20   Ron et al. (1993), J. Biol. Chem., 268:2984-2988). In
assays of tissues or organs there will be less
radioactivity from ^{125}I-KGF analog binding to KGFR, as
compared to a standardized binding curve of ^{125}I-KGF
analog, due to unlabeled native KGF binding to KGFR.
25   Similarly, the use of ^{125}I-KGF analog may be used to
detect the presence of KGFR in various cell types.

This invention also contemplates the use of a
KGF analog in the generation of antibodies made against
the peptide, which antibodies also bind to native KGF.
30   In this embodiment, the antibodies are monoclonal or
polyclonal in origin and are generated using a KGF
analog. The resulting antibodies bind preferentially to
native KGF, preferably when that protein is in its
native (biologically active) conformation. These
35   antibodies can be used for detection or purification of
the native KGF.

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        Moreover, the invention contemplates the use
of the KGF analogs in the discovery of high affinity or
low affinity KGF binding molecules having therapeutical
applications, for example, as a way for efficient KGF
5   delivery or as an inhibitor for KGF activity. The
thermal stability of the KGF analogs is important to
identify such binding molecules in physiological
conditions (i.e., at 37°C) since their affinity for KGF
could be strongly temperature-dependent and may be
10 unpredictable from the affinity observed at 4°C.

        For *in vivo* uses, the KGF analogs may be
formulated with additives. Such additives include
buffers, carriers, stabilizers, excipients,
preservatives, tonicity adjusting agents, anti-oxidants
15 and the like (*e.g.*, viscosity adjusting agents or
extenders). The selection of specific additives will
depend upon the storage form (i.e., liquid or
lyophilized) and the modes of administering the KGF
analog. Suitable formulations, known in the art, can be
20 found in *REMINGTON'S PHARMACEUTICAL SCIENCES* (latest
edition), Mack Publishing Company, Easton, PA.

        The KGF analogs may be applied in
therapeutically effective amounts to tissues
specifically characterized by having damage to or
25 clinically insufficient numbers of non-fibroblast
epithelium cells. Areas in which KGF analogs may be
successfully administered include, but are not limited
to: the stimulation, proliferation and differentiation
of adnexal structures such as hair follicles, sweat
30 glands, and sebaceous glands in patients with burns and
other partial and full-thickness injuries; accelerated
reepithelialization of lesions caused by epidermolysis
bullosa, which is a defect in adherence of the epidermis
to the underlying dermis, resulting in frequent open,
35 painful blisters which can cause severe morbidity;
preventing chemotherapy-induced alopecia and treating

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male-pattern baldness, or the progressive loss of hair
in men and women; treating gastric and duodenal ulcers;
treating inflammatory bowel diseases, such a Crohn's
disease (affecting primarily the small intestine) and
5   ulcerative colitis (affecting primarily the large
bowel); preventing or reducing gut toxicity in radiation
and chemotherapy treatment regimes through treatment
(e.g., pretreatment and/or postreatment) to induce a
cytoprotective effect or regeneration or both;
10   stimulating the production of mucus throughout the
gastrointestinal tract; inducing the proliferation and
differentiation of type II pneumocytes, which may help
treat or prevent diseases such as hyaline membrane
disease (i.e., infant respiratory distress syndrome and
15   bronchopulmonary dysplasia) in premature infants;
stimulating the proliferation and differentiation of the
bronchiolar and/or alveolar epithelium with acute or
chronic lung damage or insufficiency due to inhalation
injuries (including high oxygen levels), emphysema, use
20   of lung damaging chemotherapeutics, ventilator trauma or
other lung damaging circumstances; increasing liver
function to treat or prevent hepatic cirrhosis,
fulminant liver failure, damage caused by acute viral
hepatitis and/or toxic insults to the liver; inducing
25   corneal cell regeneration, for example in the treatment
of corneal abrasion; inducing epithelial cell
regeneration to treat progressive gum disease; inducing
regeneration of tympanic epithelial cells to treat ear
drum damage and treating or preventing the onset of
30   diabetes mellitus or as an adjunct in the setting of
islet cell transplantation.

          A patient in need of proliferation of non-
fibroblast epithelial cells will be administered an
effective amount of a KGF analog. An "effective amount"
35   is that amount of the KGF analog required to elicit the
desired response in the patient being treated and will,

thus, generally be determined by the attending physician. Factors influencing the amount of KGF analog administered will include the age and general condition of the patient, the disease being treated, etc. Typical

5 dosages will range from 0.001 mg/kg body weight to 500 mg/kg body weight.

The KGF analog may be safely administered parenterally (e.g., via IV, IT, IM, SC, or IP routes), orally or topically to warm-blooded animals (such as

10 humans). The KGF analog may be used once or administered repeatedly, depending on the disease and the condition of the patient. In some cases, the KGF analog may be administered as an adjunct to other therapy and also with other pharmaceutical preparations.

15 The following examples are included to more fully illustrate the present invention. It is understood that modifications can be made in the procedures set forth, without departing from the spirit of the invention.

20

EXAMPLES

Standard methods for many of the procedures described in the following examples, or suitable

25 alternative procedures, are provided in widely recognized manuals of molecular biology such as, for example, *Molecular Cloning*, Second Edition, Sambrook *et al.*, Cold Spring Harbor Laboratory Press (1987) and *Current Protocols in Molecular Biology*, Ausabel *et al.*,

30 Greene Publishing Associates/Wiley Interscience, New York (1990).

EXAMPLE 1: Preparation of DNA Coding for KGF and KGF Analogs

35 The cloning of the full-length human KGF gene (encoding a polypeptide with the sequence of native KGF)

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was carried out both by polymerase chain reaction (PCR) of RNA from an animal cell and by PCR of chemically synthesized (*E. coli* optimized codon) oligonucleotides ("OLIGOs"). Both procedures are described below:

5   PCR amplification using RNA isolated from cells known to produce the polypeptide was performed. Initially, cells from a human fibroblast cell line AG1523A (obtained from Human Genetic Mutant Cell Culture Repository Institute For Medical Research,

10   Camden, New Jersey) were disrupted with guanidium thiocyanate, followed by extraction (according to the method of Chomyzinski *et al.* (1987), *Anal. Biochem.*, 172:156). Using a standard reverse transcriptase protocol for total RNA, the KGF cDNA was generated.

15   PCR (PCR#1) amplification of the KGF gene was carried out using the KGF cDNA as template and primers OLIGO#1 and OLIGO#2 that encode DNA sequences immediately 5' and 3' of the KGF gene [Model 9600 thermocycler (Perkin-Elmer Cetus, Norwalk, CT); 28 cycles; each

20   cycle consisting of one minute at 94°C for denaturation, two minutes at 60°C for annealing, and three minutes at 72°C for elongation]. A small aliquot of the PCR#1 product was then used as template for a second KGF PCR (PCR#2) amplification identical to the

25   cycle conditions described above except for a 50°C annealing temperature. For expression cloning of the KGF gene, nested PCR primers were used to create convenient restriction sites at both ends of the KGF gene. OLIGO#3 and OLIGO#4 were used to modify the KGF

30   DNA product from PCR#2 to include *MluI* and *BamHI* restriction sites at the 5' and 3' ends of the gene, respectively [PCR#3; 30 cycles; each cycle consisting of one minute at 94°C for denaturation, two minutes at 60°C for annealing, and three minutes at 72°C for

35   elongation]. This DNA was subsequently cut with *MluI* and *BamHI*, phenol extracted, and ethanol precipitated.

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It was then resuspended and ligated (using T4 ligase)
into a pCFM1156 plasmid (Figure 2A) that contained a
"RSH" signal sequence to make construct RSH-KGF
(Figure 3).

5   The ligation products were transformed
(according to the method of Hanahan (1983), *J. Mol.
Biol.*, *166*:557) into *E. coli* strain FM5 (ATCC: 53911)
and plated onto LB+kanamycin at 28°C. Several
transformants were selected and grown in small liquid
10   cultures containing 20 μg/mL kanamycin. The RSH-KGF
plasmid was isolated from the cells of each culture and
DNA sequenced. Because of an internal *NdeI* site in the
KGF gene, it was not possible to directly clone the
native gene sequence into the desired expression vector
15   with the bracketed restriction sites of *NdeI* and *BamHI*.
This was accomplished as a three-way ligation. Plasmid
RSH-KGF was cut with the unique restriction sites of
BsmI and *SstI*, and a ~3 kbp DNA fragment (containing
the 3' end of the KGF gene) was isolated following
20   electrophoresis through a 1% agarose gel. A PCR
(PCR#4) was carried out as described for PCR#3 except
for the substitution of OLIGO#5 for OLIGO#3. The PCR
DNA product was then cut with *NdeI* and *BsmI* and a 311
bp DNA fragment was isolated following electrophoresis
25   through a 4% agarose gel. The third piece of the
ligation is a 1.8 kbp DNA fragment of pCFM1156 cut with
NdeI and *SstI* isolated following electrophoresis
through a 1% agarose gel. Following ligation (T4
ligase), transformation, kanamycin selection and DNA
30   sequencing as described above, a clone was picked
containing the construct in Figure 4 and the plasmid
designated KGF. Because of an internal ribosomal
binding site that produced truncated products, the KGF
DNA sequence between the unique *KpnI* and *EcoRI* sites
35   was replaced with chemically synthesized OLIGOs

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(OLIGO#6 through OLIGO#11) to minimize the use of the internal start site (Figure 5).

OLIGO#1 (SEQ ID NO:7):   5'-CAATGACCTAGGAGTAACAATCAAC-3'

5   OLIGO#2 (SEQ ID NO:8):   5'-AAAACAAACATAAATGCACAAGTCCA-3'

OLIGO#3 (SEQ ID NO:9):   5'-ACAACGCGTGCAATGACATGACTCCA-3'

OLIGO#4 (SEQ ID NO:10):

         5'-ACAGGATCCTATTAAGTTATTGCCATAGGAA-3'

OLIGO#5 (SEQ ID NO:11):

10   5'-ACACATATGTGCAATGACATGACTCCA-3'

OLIGO#6 (SEQ ID NO:12):

         5'-CTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCC-3'

OLIGO#7 (SEQ ID NO:13):

         5'-AAGAGATGAAAAACAACTACAATATTATGGAAATCCGTACTGTT-3'

15   OLIGO#8 (SEQ ID NO:14):

         5'-GCTGTTGGTATCGTTGCAATCAAAGGTGTTGAATCTG-3'

OLIGO#9 (SEQ ID NO:15):

         5'-TCTTGGGTGCCCTTGACTTTGCCGCGTTTGTCGATACGCAGGTAC-3'

OLIGO#10 (SEQ ID NO:16):

20   5'-ACAGCAACAGTACGGATTTCCATAATATTGTAGTTGTTTTTCATC-3'

OLIGO#11 (SEQ ID NO:17):

         5'-AATTCAGATTCAACACCTTTGATTGCAACGATACCA-3'

The OLIGOs were phosphorylated with T4
25   polynucleotide kinase and then heat denatured. The
single-stranded (ss) OLIGOs were then allowed to form a
ds DNA fragment by allowing the temperature to slowly
decrease to room temperature. T4 ligase was then used
to covalently link both the internal OLIGO sticky-ends
30   and the whole ds OLIGO fragment to the KGF plasmid cut
with *KpnI* and *EcoRI*. The new plasmid was designated
KGF(dsd).

A completely *E. coli* codon-optimized KGF gene
was constructed by PCR amplification of chemically
35   synthesized OLIGOs #12 through 24.

OLIGO#12 (SEQ ID NO:18):   5'-AGTTTTGATCTAGAAGGAGG-3'

OLIGO#13 (SEQ ID NO:19):   5'-TCAAAACTGGATCCTATTAA-3'

OLIGO#14 (SEQ ID NO:20):

    5'-AGTTTTGATCTAGAAGGAGGAATAACATATGTGCAACGACATG-
    ACTCCGGAACAGATGGCTACCAACGTTAACTGCTCCAGCCCGGAACGT-3'

OLIGO#15 (SEQ ID NO:21):

    5'-CACACCCGTAGCTACGACTACATGGAAGGTGGTGACATCCGT-
    GTTCGTCGTCTGTTCTGCCGTACCCAGTGGTACCTGCGTATCGACAAA-3'

OLIGO#16 (SEQ ID NO:22):

    5'-CGTGGTAAAGTTAAAGGTACCCAGGAAATGAAAAACAACTACAACATC-
    ATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCAATCAAA-3'

OLIGO#17 (SEQ ID NO:23):

    5'-GGTGTTGAATCTGAATTCTACCTGGCAATGAACAAAGAAGGTAAACT-
    GTACGCAAAAAAAGAATGCAACGAAGACTGCAACTTCAAAGAA-3'

OLIGO#18 (SEQ ID NO:24):

    5'-CTGATCCTGGAAAACCACTACAACACCTACGCATCTGCTAAATGGAC-
    CCACAACGGTGGTGAAATGTTCGTTGCTCTGAACCAGAAAGGT-3'

OLIGO#19 (SEQ ID NO:25):

    5'-ATCCCGGTTCGTGGTAAAAAAACCAAAAAAGAACAGAAAACCGCTC-
    ACTTCCTGCCGATGGCAATCACTTAATAGGATCCAGTTTTGA-3'

OLIGO#20 (SEQ ID NO:26):5'-TACGGGTGTGACGTTCCGGG-3'

OLIGO#21 (SEQ ID NO:27):5'-CTTTACCACGTTTGTCGATA-3'

OLIGO#22 (SEQ ID NO:28):5'-ATTCAACACCTTTGATTGCA-3'

OLIGO#23 (SEQ ID NO:29):5'-CCAGGATCAGTTCTTTGAAG-3'

OLIGO#24 (SEQ ID NO:30):5'-GAACCGGGATACCTTTCTGG-3'

        OLIGOs #12 through 24 were designed so
that the entire DNA sequence encoding native KGF was
represented by OLIGOs from either the "Watson" or the
"Crick" strand and upon PCR amplification would produce
the desired double-stranded DNA sequence (Figure 6)
[PCR#5, Model 9600 thermocycler, Perkin-Elmer Cetus]; 21
cycles, each cycle consisting of 31 seconds at 94°C for
denaturation, 31 seconds at 50°C for annealing, and 31
seconds at 73°C for elongation; following the 21 cycles
the PCR was finished with a final elongation step of 7

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minutes]. After PCR amplification, the DNA fragment was
cut with *XbaI* and *BamHI* and the 521 bp fragment ligated
into the expression plasmid pCFM1156 cut with the same
enzymes. PCR#5 utilized the outside primers (100

5   pmoles/100 µl rxn) OLIGO#12 and OLIGO#13 and 1 µl/100 µl
rxn of a KGF template derived by ligation (by T4 ligase)
of OLIGO#14 through OLIGO#19 (OLIGO#15 through OLIGO#18
were phosphorylated with T4 polynucleotide kinase) using
OLIGO#20 through OLIGO#24 as band-aid oligos (Jayaraman

10   *et al.* (1992), *Biotechniques*, 12:392) for the ligation.
The final construct was designated KGF
(codon optimized).

All of the KGF analogs described herein are
composed in part from DNA sequences found in KGF(dsd) or

15   KGF(codon optimized), or a combination of the two. The
sequences are further modified by the insertion into
convenient restriction sites of DNA sequences that
encode the particular KGF analog amino acids made
utilizing one or more of the above-described techniques

20   for DNA fragment synthesis. Any of the analogs can be
generated in their entirety by the above described
techniques. However, as a part of the general OLIGO
design optimized *E. coli* codons were used where
appropriate, although the presence of *E. coli* optimized

25   codons in part or *in toto* of any of the genes where
examined did not significantly increase the yield of
protein that could be obtained from cultured bacterial
cells. Figures 7 to 24 and 37 to 50 set forth by
convenient example particular KGF analog nucleotide and

30   amino acid sequence constructions: C(1,15)S (Figure 7);
ΔN3/C(15)S (Figure 8); ΔN3/C(15)- (Figure 9); ΔN8/C(15)S
(Figure 10); ΔN8/C(15)- (Figure 11); ΔN15 (Figure 12);
ΔN16 (Figure 13); ΔN17 (Figure 14); ΔN18 (Figure 15);
ΔN19 (Figure 16); ΔN20 (Figure 17); ΔN21 (Figure 18);

35   ΔN22 (Figure 19); ΔN23 (Figure 20); ΔN24 (Figure 21);
C(1,15)S/R(144)E (Figure 22); C(1,15)S/R(144)Q

(Figure 23); ΔN23/R(144)Q (Figure 24); C(1,15,40)S
(Figure 37); C(1,15,102)S (Figure 38); C(1,15,102,106)S
(Figure 39); ΔN23/N(137)E (Figure 40); ΔN23/K(139)E
(Figure 41); ΔN23/K(139)Q (Figure 42); ΔN23/R(144)A
(Figure 43); ΔN23/R(144)E (Figure 44); ΔN23/R(144)L
(Figure 45); ΔN23/K(147)E (Figure 46); ΔN23/K(147)Q
(Figure 47); ΔN23/K(153)E (Figure 48); ΔN23/K(153)Q
(Figure 49) and ΔN23/Q(152)E/K(153)E (Figure 50). All
the KGF analog constructions described herein were DNA
sequence confirmed.

EXAMPLE 2: Production in *E. coli*

Three different expression plasmids were
utilized in the cloning of the KGF analog genes. They
were pCFM1156 (ATCC# 69702), pCFM1656 (ATCC# 69576), and
pCFM3102 (Figures 2A, 2B and 2C, respectively). The
plasmid p3102 can be derived from the plasmid pCFM1656
by making a series of site-directed base changes with
PCR overlapping oligo mutagenesis. Starting with the
BglII site (pCFM1656 plasmid bp # 180) immediately 5' to
the plasmid replication promoter, PcopB, and proceeding
toward the plasmid replication genes, the base pair
changes are as follows:

pCFM1656 bp #	bp in pCFM1656	bp changed to in pCFM3102
# 204	T/A	C/G
# 428	A/T	G/C
# 509	G/C	A/T
# 617	- -	insert two G/C bp
# 677	G/C	T/A
# 978	T/A	C/G
# 992	G/C	A/T
# 1002	A/T	C/G
# 1005	C/G	T/A
# 1026	A/T	T/A
# 1045	C/G	T/A
# 1176	G/C	T/A
# 1464	G/C	T/A
# 2026	G/C	bp deletion
# 2186	C/G	T/A
# 2479	A/T	T/A
# 2498-2501	AGTG TCAC	GTCA CAGT
# 2641-2647	TCCGAGC AGGCTCG	bp deletion
# 3441	G/C	A/T
# 3452	G/C	A/T
# 3649	A/T	T/A
# 4556	--	insert bps

(SEQ ID NO:67) 5'-GAGCTCACTAGTGTCGACCTGCAG-3'
(SEQ ID NO:68) 3'-CTCGAGTGATCACAGCTGGACGTC-5'

As seen above, pCFM1156, pCFM1656 and pCFM3102 are very similar to each other and contain many of the same restriction sites. The plasmids were chosen by convenience, and the vector DNA components can be easily exchanged for purposes of new constructs. The host used for all cloning was *E. coli* strain FM5 (ATCC: 53911) and the transformations were carried out (according to the method of Hanahan (1983), *supra*) or by electroelution with a Gene Pulser™ transfection apparatus (BioRad Laboratories, Inc., Hercules, CA) according to the manufacturer's instructions.

Initially, a small, freshly cultured inoculum of the desired recombinant *E. coli* clone harboring the

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desired construct on one of the three pCFM vectors was
started by transferring 0.1 mL of a frozen glycerol
stock of the appropriate strain into a 2 L flask
containing 500 mL of Luria broth. The culture was
5   shaken at 30°C for 16 hours, after which the culture was
transferred to a 15 L fermentor containing 8 L of
sterile batch medium (Tsai, et al. (1987), *J. Industrial
Microbiol.*, 2:181-187).

Feed batch fermentation starts with the
10   feeding of Feed # 1 medium (Tsai, et al. (1987),
supra). When the OD600 reached 35, expression of the
desired KGF analog was induced by rapidly raising the
culture temperature to 37°C for two hours then up to .
42°C to denature the CI repressor. The addition of Feed
15   1 was discontinued in favor of Feed 2, the addition rate
of which was initiated at 300 mL/hr. Feed 2 comprised
175 g/L trypticase-peptone, 87.5 g/L yeast extract, and
260 g/L glucose. After one hour at 42°C, the culture
temperature was decreased to 36°C, where this
20   temperature was then maintained for another 6 hours.

The fermentation was then halted and the cells
were harvested by centrifugation into plastic bags
placed within 1 L centrifuge bottles. The cells were
pelleted by centrifugation at 400 rpm for 60 minutes,
25   after which the supernatants were removed and the cell
paste frozen at -90°C.

Following expression of the various KGF
analogs in *E. coli*, native KGF, C(1,15)S,
C(1,15)S/R(144)E, C(1,15)S/R(144)Q, ΔN15, ΔN23, and
30   ΔN23/R(144)Q proteins were purified using the following
procedure. Cell paste from a high cell density
fermentation was suspended at 4°C in 0.2 M NaCl, 20 mM
NaPO$_4$, pH 7.5 as a 10-20% solution (weight per volume)
using a suitable high shear mixer. The suspended cells
35   were then lysed by passing the solution through a
homogenizer (APV Gaulin, Inc., Everett, MA) three times.

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The outflowing homogenate was cooled to 4-8°C by using a
suitable heat exchanger. Debris was then removed by
centrifuging the lysate in a J-6B™ centrifuge (Beckman
Instruments, Inc., Brea, CA) equipped with a JS 4.2

5   rotor at 4,200 rpm for 30-60 min. at 4°C. Supernatants
were then carefully decanted and loaded onto a
previously prepared 450 mL (5 cm x 23 cm) column of S-
Sepharose Fast Flow™ resin (Pharmacia, Piscataway, NJ)
equilibrated with 0.2 M NaCl, 20 mM $NaPO_4$, pH 7.5 at

10   4°C. Next, the column was washed with five column
volumes (2250 mL) of 0.4 M NaCl, 20 mM $NaPO_4$, pH 7.5 at
4°C. The desired protein was eluted by washing the
column with 5 L of 0.5 M NaCl, 20 mM $NaPO_4$, pH 7.5.
50 mL fractions were collected and the A_{280} of the

15   effluent was continuously monitored. Fractions
identified by A_{280} as containing eluted material were
then analyzed by SDS-PAGE through 14% gels to confirm
the presence of the desired polypeptide.
        Those fractions containing proteins of

20   interest were then pooled, followed by the addition of
an equal volume of distilled water. The diluted sample
was then loaded onto a previously prepared a 450 mL
(5 cm x 23 cm) column of S-Sepharose Fast Flow
equilibrated with 0.4 M NaCl, 20 mM $NaPO_4$, pH 6.8 at

25   4°C. The column was washed with 2250 mL of 0.4 M NaCl,
20 mM $NaPO_4$, pH 6.8 and the protein eluted using a 20
column volume linear gradient ranging from 0.4 M NaCl,
20 mM $NaPO_4$, pH 6.8 to 0.6 M NaCl, 20 mM $NaPO_4$, pH 6.8.
Again, 50 mL fractions were collected under constant A_{280}

30   monitoring of the effluent. Those fractions containing
the protein (determined by 14% SDS-PAGE) were then
pooled, followed by concentration through a YM-10
membrane (10,000 molecular weight cutoff) in a 350cc
stirring cell (Amicon, Inc. Mayberry, MA) to a volume of

35   30-40 mL.

The concentrate was then loaded onto a
previously generated 1,300 mL (4.4 cm x 85 cm) column of
Superdex-75™ resin (Pharmacia) equilibrated in column
buffer comprising 1X PBS (Dulbecco's Phosphate Buffered
5   Saline, "D-PBS", calcium and magnesium-free) or 0.15 M
NaCl, 20 mM NaPO$_4$, pH 7.0. After allowing the sample to
run into the column, the protein was eluted from the gel
filtration matrix using column buffer. Thereafter,
10 mL fractions were recovered and those containing the
10   analog (determined by 14% SDS-PAGE) were pooled.
Typically, the protein concentration was about 5-10
mg/mL in the resultant pool. All of the above
procedures were performed at 4-8°C, unless otherwise
specified.

15   An alternative purification procedure was used
to purify native KGF, C(1,15)S and ΔN23. The procedure
involves the following steps, and unless otherwise
specified, all procedures, solutions and materials were
conducted at 23 ± 5°C.

20   Upon completion of the production phase of a
bacterial fermentation, the cell culture was cooled to
4-8°C and the cells harvested by centrifugation or a
similar process. On the basis of the expected yield of
protein per unit weight of cell paste and the amount of
25   purified protein required, an appropriate amount of cell
paste, by weight, was suspended in a mild buffer
solution of 20 mM NaPO$_4$, 0.2 M NaCl, pH 7.5, weighing
about five times that of the cell paste to be suspended.
The cells were dispersed to a homogeneous solution using
30   a high shear mixer. The temperature of the cell paste
dispersion was maintained at 4-8°C during
homogenization.

             The cells were then lysed by pressure, for
example by passing the cell paste dispersion twice
35   through an appropriately-sized cell homogenizer. The
homogenate was kept chilled at 5 ± 3°C. To clarify the

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cell lysate, a previously prepared depth filter housing
(Cuno, Inc., Meriden, CT) equipped with a filter having
an appropriate amount of filter surface area,
equilibrated with a suitable volume of 0.2 M NaCl, 20 mM
5   NaPO₄, pH 7.5, was employed. The equilibration and
clarification were performed at 5 ± 3°C. Prior to
clarification, an appropriate amount of a suitable
filter aid was used to pre-coat the filter and be
thoroughly mixed with the cell lysate, after which the
10 lysate was clarified by passing the solution through the
filter apparatus. The filter was washed with 0.2 M
NaCl, 20 mM NaPO₄, pH 7.5. The filtrate and any
subsequent wash were collected in a chilled container of
suitable capacity, all the while being maintained at
15 less than 10°C.

Following clarification, the lysate was then
passed through a previously prepared a column of
SP-Sepharose Fast Flow containing at least 1 mL of resin
per 2 g of cell paste. The column of SP-Sepharose Fast
20 Flow was equilibrated with cold (5 ± 3°C), 0.2 M NaCl,
20 mM NaPO₄, pH 7.5. The temperature of the column was
maintained at less than 10°C. The clarified lysate
(5 ± 3°C) was then loaded onto the ion exchange column,
with the absorbance at 280 nm (A$_{280}$) of eluate being
25 continuously monitored. After sample loading, the
column was washed with cold 0.2 M NaCl, 20 mM NaPO₄, pH
7.5, followed by washing with 0.3 M NaCl, 20 mM NaPO₄,
pH 7.5 at 23 ± 5°C.

To elute the desired protein, a linear
30 gradient ranging from 0.2-1 M NaCl, 20 mM NaPO₄, pH 7.5
was used. Bulk product was collected in several
fractions on the basis of the A$_{280}$ of the eluate.
Following elution, these fractions were pooled and the
volume noted.

35 To oxidize free sulfhydryl groups, an
oxidation step was performed. For proteins with altered

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cysteine patterns, as compared to native KGF, an
oxidizing agent (e.g., cystamine dihydrochloride or
another appropriate oxidizing agent, for instance,
cystine, oxidized glutathione or divalent copper) was
5   added to a final concentration of 1-20 mM and the pH was
adjusted to 7-9.5, with a pH of 9.0 ± 0.3 being
preferred when cystamine dihydrochloride was used. The
oxidation was conducted at 10-30°C for an appropriate
period. For the native KGF protein, oxidation was
10 accomplished by adding an appropriate amount of $(NH_4)_2SO_4$
such as 1-2 M $(NH_4)_2SO_4$, adjusting the pH to 7.5 ± 9.5,
and holding the temperature at 23 ± 5°C for an
appropriate period.

After oxidation, the pH of the solution was
15 adjusted to between 6.5 and 9.5. If necessary, solid
$(NH_4)_2SO_4$ was added to the solution to a final
concentration of 2 M. To remove particulates, the
solution was passed through appropriate clarification
filters.
20 The filtered, oxidized product was then
subjected to hydrophobic interaction chromatography
(HIC). The HIC matrice was Butyl-650M Toyopearl™ resin
(Tosohaas, Inc., Montgomeryville, PA). The protein-
containing solution was loaded onto the column, which
25 had been previously equilibrated with 2 M $(NH_4)_2SO_4$, 0.15
M NaCl, 20 mM NaPO$_4$, pH 7.0. After sample loading, the
column was washed with 2 M $(NH_4)_2SO_4$, 0.15 M NaCl, 20 mM
NaPO$_4$, pH 7.0. The desired protein was then eluted
using a decreasing linear $(NH_4)_2SO_4$ gradient ranging from
30 2-0 M developed in 0.15 M NaCl, 20 mM NaPO4, pH 7.0.
When the desired protein began to elute, as indicated by
an increase in the A$_{280}$ of the eluate, fractions were
collected. Aliquots of each fraction were then analyzed
by SDS-PAGE. Those fractions containing the desired
35 protein were then pooled, thoroughly mixed, and the

volume of the pool determined, as was the concentration of the protein therein.

5   The pooled HIC protein-containing eluate was then concentrated and the elution buffer exchanged. Typically, proteins were concentrated to 5.0-10.0 mg/mL. Ultrafiltration was conducted using an ultrafiltration system equipped with a PTGC Pellicon™ cassette system (Millipore, Inc., Bedford, MA) with an appropriately sized cut-off membrane

10   After concentration, the sample was diafiltered against an appropriate buffer. The retentate from the concentration step was diafiltered against 0.15 M NaCl, 20 mM NaPO$_4$, pH 7.0 until the conductivity of the retentate was within 5% of the

15   conductivity of the 0.15 M NaCl, 20 mM NaPO$_4$, pH 7.0 solution.

In addition, to remove precipitates and bacterial endotoxin that might be present, the concentrated diafiltered protein-containing sample was

20   passed through a 0.1 μm Posidyne™ filter (Pall, Inc., Cortland, NY). After determining the protein concentration of the solution and on the basis of the desired concentration of the final bulk product, the solution was diluted with 0.15 M NaCl, 20 mM sodium

25   phosphate, pH 7.0, to the desired final concentration. A final aseptic filtration through a 0.22 μm filter was then performed as the final bulk product was transferred to a pyrogen-free container for storage (at about 5°C) for further formulation.

30

B.   Analysis

Analysis was conducted using *E. coli*-derived, native KGF; C(1,15)S; C(1,15)S/R(144)Q; ΔN15; ΔN23 and

35   ΔN23/R(144)Q.

Conformational Stability

The polypeptides were compared by their
storage stability, thermal unfolding transition
5   temperatures (T_m), and stability in a broad range of pH
conditions.

Storage Stability

10   Native KGF and C(1,15)S were incubated at 37°C
for about 24 hrs, and the remaining soluble protein
determined. The results are summarized in Figures 24 to
26.

Figure 25 compares native KGF and C(1,15)S
15   when stored in 20 mM Na phosphate, 0.15 M NaCl, pH 7.0
at 37°C for 27 hrs. C(1,15)S showed a significantly
increased amount of soluble protein relative to native
KGF.

Figure 26 compares native KGF and C(1,15)S
20   stored in 0.15 M NaCl, 20 mM NaPO$_4$, 0.15 M NaCl, pH 7.0
when stored at 37°C. Here again, C(1,15)S gave enhanced
stability relative to native KGF.

Figure 27 compares the stability of native
KGF, ΔN15 and C(1,15)S in PBS and in 50 mM NaPO$_4$, 0.15 M
25   NaCl, pH 7.0, when stored at 37°C for 18 hrs. The
recovery of soluble protein is much higher in high
phosphate than in PBS (100 vs. 65%). ΔN15 as well as
C(1,15)S showed significantly enhanced stability, as
compared with native KGF. ΔN15 and C(1,15)S also gave
30   ~100% recovery in high phosphate, as expected from the
result of native KGF.

However, a single preliminary comparative
example of storage stability was conducted between
C(1,15,40)S, and C(40)S (which is encoded by bases 201
35   to 684 of SEQ ID NO:1, except that Ser[40] is encoded by
AGA); and between C(1,15,102), and C(102)S (which is

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encoded by bases 201 to 684 of SEQ ID NO:1, except that
Ser[102] is encoded by AGG). The results (not shown)
indicated a decreased stability (i.e., less soluble
protein after being stored at 37° C). However, the

5 amount of soluble, correctly folded C(1,15,40)S protein
which was purified from the culture medium was greater
than that of C(40)S, suggesting that C(1,15,40)S may in
fact be more stable and that the results from the
comparative example are inconclusive. The present

10 invention preferably includes a KGF analog having other
than substitutions at Cys[40], Cys[102], Cys[106] and more
preferably excludes C(1,15,40)S, C(1,15,102)S and
C(1,15,40,102,106)

        The ability of C(1,15)S/R(144)Q and

15 ΔN23/R(144)Q to prevent aggregation at elevated
temperatures was also examined. Samples containing 0.5
mg/mL of protein were prepared in D-PBS. 0.5 mL of each
sample was aliquoted into 3 cc type-1 glass vials. The
vials were sealed with rubber stoppers and 13 mm flip-
off aluminum seals were crimped on. These vials were

20 then placed in a 37°C incubator. At predetermined time
intervals, vials were withdrawn and analyzed for the
loss of soluble protein. Visible precipitates were
removed by centrifuging 250 μL of each sample through a

25 0.22 μm Spin-X™ filter unit (Costar, Cambridge, MA).
Soluble protein in the filtered solutions was
subsequently analyzed by size exclusion HPLC. The
amount of soluble protein was determined by integrating
the HPLC peak area and plotting the result as a function

30 of incubation time at 37°C. The results are shown in
Figure 27.
        The half-lives for the loss of soluble,
monomeric protein were then estimated from these kinetic
curves. Table 1 shows the half-life for remaining

35 soluble KGF upon storage at 37°C for these proteins.

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Table 1

<u>Half-life for the Loss of Soluble, Monomeric Proteins</u>

Protein	t1/2 (day)
native KGF	0.6
ΔN23	1.1
C(1,15)	1.2
C(1,15)S/R(144)Q	13.3
ΔN23/R144Q	22.3
C(1,15)S/R(144)E	38.0

5   As seen in Table 1, above, and Figure 28, the
native KGF aggregated the most rapidly, with a
solubility half-life of 0.6 days. C(1,15)S/R(144)Q,
ΔN23/R(144)Q and C(1,15)S/R(144)E showed substantial
increases in the solubility half-life to 13.3, 22.3 and
10   38 days, respectively.

<u>Thermal Unfolding</u>

           Thermal unfolding was monitored by circular
15   dichroism (CD) at 230 nm using a J-720™
spectropolarimeter (Jasco, Inc., Easton, MD) equipped
with a PTC-343 Peltier-type temperature control system.
For CD analysis, separate samples containing 0.1 mg/mL
of the polypeptide to be analyzed were prepared in D-PBS
20   (Life Technologies, Inc., Grand Island, NY). For each
sample, about 2.5 mL was loaded into a 10 mm path length
rectangular Suprasil™ quartz (Heraeus Quarzschmelze,
GmbH, Hanau, Germany) fluorescent cell (Hellma Cells,
Inc., Jamaica, NY). The cell was then placed into the
25   Peltier-type temperature control system in the
spectropolarimeter.   Thermal unfolding was carried out
at a rate of 50°C/hr. Changes in ellipticity were
monitored at 230 nm to indicate unfolding. The T_m of
each sample was estimated by identifying a temperature

at which 50% of protein molecules in the solution were
unfolded (*Biophysical Chemistry*, Cantor and Schimmel
(eds), W.H. Freeman and Co. San Francisco (1980). The
estimated T_m for each of the three proteins is listed in
5   Table 2.

<div align="center">

Table 2

Estimated Melting Temperatures

</div>

Protein	T_m (°C)
native KGF	54.0
ΔN15	55.0
C(1,15)S	55.0
ΔN23	56.0
C(1,15)S/R(144)Q	62.5
ΔN23/R144Q	63.0
C(1,15)S/R(144)E	63.5

10

As these results show, C(1,15)S and ΔN15 have
a 1°C increase in the T_m as compared with native KGF.
The ΔN23 has an additional degree increase in Tm.
However, the substitution of R144Q to C(1,15)S/R(144)Q
15   or ΔN23 adds a greater than 6°C increase in T_m and more
than 7°C as compared with native KGF. Moreover,
C(1,15)S/R(144)E is greater than 9°C more stable than
native KGF.

20   **pH**

The acid stabilities of C(1,15)S/R(144)Q and
C(1,15)S/R(144)E were also compared to that of native
KGF, by adjusting D-PBS to different pH values by adding
25   concentrated HCl or NaOH. Approximately 2.35 mL of
D-PBS at different pH values was mixed with 100 µL of
2.45 mg/mL KGF protein in a quartz cell. These samples

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were thermally unfolded at a rate of 50°C/hr and
monitored by CD at 230 nm. Figure 29 shows the T_m as a
function of pH for native KGF, C(1,15)S/R(144)Q and
C(1,15)S/R(144)E. In the pH range tested, the
5   C(1,15)S/R(144)Q and C(1,15)S/R(144)E always have a
higher T_m than the native KGF.

In vitro Biological Activity

10   In vitro mitogenic activity of C(1,15)S, ΔN15,
ΔN23, ΔN23/R(144)Q, C(1,15)S/R(144)Q and
C(1,15)S/R(144)E was also determined as a function of
protein concentration and the half-maximal
concentrations by measurement of [^{3}H]-thymidine uptake
15   by Balb/MK cells (according to the methods of Rubin et
al. (1989), supra). Generally, the concentrations of
each of the KGF analogs relative to a known standard
native KGF was determined using an in vitro biological
assay. Each KGF analog was then diluted and assayed for
20   biological activity using a Balb/MK mitogenic assay.
The samples were first diluted in a bioassay medium
consisting of 50% customer-made Eagle's MEM, 50%
customer-made F12, 5 µg/ml transferrin, 5 ng/ml sodium
selenite, 0.0005% HSA and 0.005% Tween 20. KGF samples
25   were then added into Falcon primeria 96-well plates
seeded with Balb/MK cells. Incorporation of [^{3}H]-
Thymidine during DNA synthesis was measured and
converted to input native KGF concentration by
comparison to a native KGF standard curve. The results
30   are presented in Figures 30 to 35. As seen in the
Figures, the tested KGF analogs described in Figures 29
to 34 have comparable activity to native KGF.

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Example 2:   Production in Mammalian Cell Culture

            This example describes the expression,
isolation, and characterization of two biologically
5   active recombinant KGF (rKGF) forms produced in a
mammalian expression system.
            The human KGF gene was isolated by PCR
amplification of cDNA made from normal dermal human
fibroblast cells (Clonetec, Inc., San Diego, CA).
10 Following the making of cDNA by reverse transcriptase,
PCR was used to amplify the KGF gene. OLIGO#25 and
OLIGO#26 were used to amplify the gene out of the cDNA,
and OLIGO#27 and OLIGO#28 were used to place HindIII and
BglII restriction sites at the fragment ends by a second
15 PCR amplification, as set forth in Figure 1.

        OLIGO#25 (SEQ ID NO:69):   5'-CAATCTACAATTCACAGA-3'

        OLIGO#26 (SEQ ID NO:70):   5'-TTAAGTTATTGCCATAGG-3'

        OLIGO#27 (SEQ ID NO:71):   5'-AACAAAGCTTCTACAATTCACAGATAGGA-3'

20   OLIGO#28 (SEQ ID NO:72):   5'-AACAAGATCTTAAGTTATTGCCATAGG-3'

            Following cloning and DNA sequence
confirmation, the KGF gene DNA was then used.
Amplification was effected using OLIGO#29 and OLIGO#30.

25

        OLIGO#29 (SEQ ID NO:73):

                    5'-CGGTCTAGACCACCATGCACAAATGGATACTGACATGG-3'

        OLIGO#30 (SEQ ID NO:74):

                    5'-GCCGTCGACCTATTAAGTTATTGCCATAGGAAG-3'

30

            The sense primer, OLIGO#29, included an XbaI
site and a consensus Kozak translation sequence (5'-
CCACC-3') upstream of the start codon, ATG. The
antisense primer, OLIGO#30, included a SalI cloning site
35 and an additional stop codon. After 18 cycles of PCR
amplification (30 sec. denaturation at 94°C, 40 sec.

annealing at 55°C, and 40 sec. elongation at 72°C), the product was digested with *Xba*I and *Sal*I and ligated with a similarly digested DNA of pDSRα2 (according to the methods of Bourdrel *et al.* (1993), *Protein Exp. & Purif.*, $\underline{4}$:130-140 and Lu *et al.* (1992), *Arch. Biochem. Biophys.*, $\underline{298}$:150-158). This resulted in plasmid KGF/pDSRα2 which placed the human KGF gene between the SV40 early promoter and the α-FSH polyadenylation sequences. Two clones were picked and DNA sequence analysis confirmed construction of the desired vector.

Two micrograms of KGF/pDSRα2 DNA were then linearized with *Pvu*I. Chinese hamster ovary (CHO) cells, seeded the day before at 0.8×10^6 cells/60 mm culture dish, were then transfected with the treated DNA using a standard calcium phosphate precipitation method (Bourdrel *et al.*, *supra*). Two weeks later, individual colonies were picked and transferred into 24-well plates. The conditioned media was considered serum free when the cells reached confluency and aliquots thereof were analyzed by Western blotting using a polyclonal rabbit antiserum reactive against *E. coli*-expressed human KGF.

Westerns were performed by running samples through 12.5% (w/v) SDS polyacrylamide gels, followed by electroblotting for 1 hr. at 400 mA onto nitrocellulose membranes using a semidry transfer apparatus (Hoefer Scientific Instruments, San Francisco, CA). 20 mM Tris, 150 mM glycine, 20% methanol served as the transfer buffer. The nitrocellulose sheets were blocked by incubation with 10% normal goat serum in PBS. Rabbit anti-serum raised against *E. coli*-derived KGF was used as primary antibody. For use, it was diluted 1/10,000 in 1% normal goat serum in PBS and incubated with the blocked nitrocellulose sheets for 12 hr. at room temperature, after which excess antibody was removed by three 30 min. washes in PBS. The nitrocellulose

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membranes were then incubated in 100 mL of 1% normal goat serum in PBS containing Vectastain™ biotinylated goat anti-rabbit IgG (secondary antibody, Vector Labs, Burlingame, CA) for 30 minutes at room temperature.

5   After three 10-minute washes in PBS, a 30-minute room temperature incubation was performed in a 100 mL solution of 1% normal goat serum containing streptavidin and biotinylated peroxidase, prepared according to manufacturer's directions (Vector Labs). Following

10   three washes in PBS, KGF cross-reactive material was visualized by incubation in a mixture of 60 µL of 30% (w/v) H_2O_2 in 100 mL of PBS and 50 mg of 4 chloronapthol in 20 mL of methanol. The reaction was stopped by rinsing in water after 10 minutes.

15   Analysis of the blots revealed that the KGF-specific antibody associated with three distinct protein bands, two being closely related with molecular weights of about 25-29 kDa and one with an estimated molecular weight of about 17 kDa, as compared to the expected

20   molecular weight of approximately 18.8 of the 163 amino acid mature protein. Additionally, several high-expressing clones secreting more than 2.0 mg of rKGF per liter, as judged by Western analysis, were selected and expanded into roller bottles (according to

25   the method of Lu et al., supra) to generate large volumes of serum-free conditioned medium for purification of KGF by cationic exchange chromatography and gel filtration, as set forth below.

             KGF from 3 L of serum-free conditioned medium

30   was purified applying the medium directly to a cation exchange column (5 x 24 cm) packed with 450 mL of sulfoethyl a column of S-Sepharose Fast Flow (Pharmacia) pre-equilibrated with 20 mM sodium phosphate, pH 7.5. After washing with five column volumes of 20 mM sodium

35   phosphate, 0.2 M NaCl, pH 7.5, rKGF was eluted using a 20-column volume linear gradient of 0.2 to 1.0 M NaCl in

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20 mM sodium phosphate, pH 7.5.   50 mL fractions were
collected with continuous A_{280} monitoring.   KGF protein
was detected by analyzing aliquots of each fraction by
SDS-PAGE.   SDS-PAGE was performed on an electrophoresis
5   system (Novex, San Diego, CA) using precast 14% Tris-
glycine precast gels (according to the method of Laemmli
(1970) *Nature*, 227:680-685).   Samples were mixed with
non-reducing SDS sample buffer without heating before
loading.   The proteins were detected by either Coomassie
10   blue or silver staining.   Two late-eluting peaks were
seen to contain protein bands corresponding to the 25-29
kDa and 17 kDa bands detected by Western blot.   The
fractions containing each of these peaks were separately
concentrated to a volume of less than 1.0 mL and
15   subjected to gel filtration.

The gel filtrations employed columns of
Superdex-75™ resin (HR 10/30, Pharmacia) pre-
equilibrated with PBS, pH 7.2, and calibrated with the
following known molecular weight standards (BioRad, San
20   Francisco, CA):   thyroglobulin (670 kDa), g-globulin
(158 kDa), ovalbumin (44 kDa), myoglobin (17 kDa) and
vitamin B-12 (1.4 kDa).   These purification steps
resulted in an approximate 2000-fold purification of
rKGF, specifically including a 17 kDa and a 30 kDa
25   material, as estimated by silver staining.

In the instance of the higher molecular weight
material, rKGF eluted as a major symmetrical peak
(by A_{280}) which was called KGF-a.   Upon SDS-PAGE analysis
of a lesser amount of this material, 3 μg/lane versus 6
30   μg/lane, two bands with a 1-2 kDa molecular weight
difference were resolved.   In the instance of the lower
molecular weight material, termed KGF-b, gel filtration
resulted in a protein preparation having the expected
mobility.   For both KGF-a and KGF-b, the overall yield
35   after purification was approximately 30-40%.

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Amino acid sequences from KGF-a and KGF-b were also analyzed. These analyses were performed on an automatic sequencer (Model 477A or 470A, Applied Biosystems, Inc., Foster City, CA) equipped with a Model

5   120A on-line PTH-amino acid analyzer and a Model 900A data collection system (according to the method of Lu et al. (1991), J. Biol. Chem., $\underline{266}$:8102-8107). Edman sequence analysis of KGF-a revealed a major N-terminal sequence of X_1-N-D-M-T-P-E-Q-M-A-T-N-V-X_2-X_3-S- [SEQ ID

10   NO:75]. A minor sequence starting from the third N-terminal amino acid, aspartic acid, was also present in 1.6% of the total sequenceable protein. X_1, X_2, and X_3 were the unassigned due to the absence of phenylthiohydantoinyl (PHT) amino acid signals during

15   sequence analysis. An N-terminal amino acid sequence of KGF predicted from cDNA sequence indicates that X_1 and X_3 are Cys residues and X_2 is asparagine. The absence of X_1 and X_3 indicates that these cysteines may form disulfide bridges. On the basis of the consensus N-linked

20   glycosylation sequence Asn-X-Ser, the absence of the predicted Asn residue corresponding to X_2 indicates that it is a potential N-linked glycosylation site.

Interestingly, N-terminal sequence analysis of KGF-b revealed an N-terminal amino acid sequence of

25   S-Y-D-Y-M-E-G-G-D-I-R-V- (SEQ ID NO:76), indicating that it is an N-terminally truncated form of KGF that has been proteolytically cleaved at the Arg^{23}-Ser^{24} peptide bond.

To further characterize purified KGF-a and

30   KGF-b, the protein was subjected to glycosidases (neuraminidase, O-glycanase, and/or N-glycanase), using known techniques (Sasaki et al. (1987), J. Biol. Chem., $\underline{262}$:12059-12076; Takeuchi et al. (1988), J. Biol. Chem., $\underline{263}$:3657-3663; Zsebo et al. (1990), Cell, $\underline{63}$:195-201).

35   These data indicate that KGF-a contains N- and O-linked carbohydrates, although the lower molecular weight form

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of KGF-a probably contains only N-linked sugar.
Glycosidase treatment did not cause molecular weight
reduction for KGF-b, indicating that the molecule is
unglycosylated.

5   The glycosylation pattern of KGF-a was further
characterized by mass spectroscopy of the endoproteinase
Glu-C-generated peptides described above. Elucidation
of carbohydrate structure of glycopeptides by the
stepped orifice method of mass spectrometric analysis
10   has been successfully applied to other proteins
(Huddleston et al. (1993), Anal. Chem., 65:877-884; Carr
et al. (1993), Prot. Sci., 2:183-196). As confirmed by
the isolation of a non-glycosylated peptide, Thr^{22} seems
to be partially glycosylated. Similar mass
15   spectrometric analysis of Asn^{14} suggested
microheterogeneity in glycosylation, with bi, tri and
tetra-antennary structures with varying degrees of
sialylation.

         Table 3A summarizes the KGF concentration to
20   stimulate [^{3}H]-thymidine incorporation of keratinocytes
at half-maximal rate (according to the method of Rubin
et al. (1989), Supra). Interaction with the KGF
receptor was examined using isolated KGF receptor
membrane preparations prepared from Balb/MK mouse
25   epidermal keratinocytes (by the procedure described by
Massague (19932), J. Biol. Chem., 258:13614-13620).
Specifically, various forms of KGF were diluted with 50
mM Tris-HCl, pH 7.5, containing 0.2% bovine serum
albumin so as to range in concentration from 0.8 ng to
30   100 ng per 50 μL. They were individually incubated with
the membrane preparation (75 ng/mL) and ^{125}I-labeled E.
coli-derived KGF (1.5 ng). Receptor binding and
competition experiments were performed at 4˚C for 16
hr., after which time samples were taken, centrifuged,
35   and washed twice with the above diluent buffer to remove
unbound and non-specifically bound, labeled KGF.

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Samples were then counted for the remaining
radioactivity.   Competition curves for receptor binding
between KGF samples and labeled KGF were constructed by
plotting percent uncompetition versus concentrations of
5   each KGF sample.   Table 3B summarizes the KGF
concentration needed to achieve 60% uncompetition
against the labeled *E. coli* derived KGF, expressed as
ng/mL.

10   Table 3A
KGF concentration to stimulate [^{3}H]-thymidine
incorporation of keratinocytes at half-maximal rate

Forms	ng/mL
E. coli KGF	10
KGF-a	30
KGF-b	30

15   Table 3B
KGF concentration to compete receptor binding
with I^{125}-labeled KGF at 60% uncompetition rate

Forms	ng/mL
E. coli KGF	65.8
KGF-a	93.5
KGF-b	89.1

20   As shown in Table 3A, KGF-a and KGF-b
stimulate comparable [^{3}H]-thymidine incorporation, with
the half-maximal rate being stimulated by approximately
30 ng/ml of either analog.   Thus, the truncation does
not reduce biological activity of the molecule.
25   However, the two analogs have approximately 3-fold lower
activity than the *E. coli*-derived, full-length KGF.   As
shown in Table 3B, radioreceptor assay uncompetition

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experiments indicated that *E. coli*-derived KGF, KGF-a, and KGF-b have similar receptor binding activity.

Example 4: *In Vivo* Biological Assay of KGF Polypeptides
5   Produced in *E. coli* and Mammalian Cell Culture

A single subcutaneous dose of KGF has been shown to result in a dose-dependent rise in serum cholesterol in mice within 24 hours. Native KGF, KGF-a,
10   C(1,15)S, ΔN15 and ΔN23 were also evaluated for the ability to raise the serum cholesterol level in a dose-dependent manner.

Each treatment group contained five female Balb/c mice (18-20 gms) obtained from CRL. The protein
15   was diluted with 0.9% saline to achieve a final injection volume of 100 μl/mouse. Each mouse was administered a single subcutaneous injection, at the following doses:

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Group	Treatment	Dose (mg/kg)
1	Native KGF	0.1
	Native KGF	0.25
	Native KGF	0.5
	Native KGF	1
	Native KGF	5
2	C(1,15)S	0.1
	C(1,15)S	0.25
	C(1,15)S	0.5
	C(1,15)S	1
	C(1,15)S	5
3	ΔN15	0.25
	ΔN15	0.5
	ΔN15	1
	ΔN15	5
4	ΔN23	0.1
	ΔN23	0.5
	ΔN23	1
	ΔN23	5
5	KGF-a	0.01
	KGF-a	0.05
	KGF-a	0.1
	KGF-a	0.5
6	Saline Control	-

Twenty-four hours after injection, the mice were sacrificed and bled via cardiac puncture. Blood samples were processed for the determination of serum
5 cholesterol.

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As shown in Figure 35, each tested KGF analog was found to raise serum cholesterol in a dose dependent manner. Also, there was no apparent difference in bioactivity between any of the tested KGF analogs.

5

In Vivo Model of Diabetes

Chemically-induced diabetes mellitus models in various animal species have been classically used to

10 study the disease and its treatment. Streptozotocin induces diabetes in the mouse, rat, hamster, dog, and monkey although studies in rats and mice are utilized most. Junod *et al.*, *Proc. Soc. Exp. Pio. Med.* **126**:210-205 (1967); Rerup, *Pharm. Rev.* **22**:485-518 (1970); Rossini

15 *et al.*, P.N.A.S. **74**:2485-2489 (1977); and *Ar'Rajab and Ahren, Pancreas* **8**:50-57 (1993). In rats, doses of streptozotocin from 45 to 70 mg/kg as a single intravenous dose induce stable disease. Doses below 45 mg/kg induce a transient disease state which is

20 reversible. Within one day of streptozotocin injection, the hyperglycemic state is induced. Blood insulin levels remain essentially unchanged compared with normal rats; however, the total content of insulin and C-peptide in the pancreas is severely decreased. Rats manifest the

25 classic signs and symptoms of diabetes in humans: increased blood glucose levels (hyperglycemia), glucose in the urine (glucosuria), increased thirst (polydipsia), increased urination (polyuria), increased appetite (hyperphagia).

30 The studies described in this disclosure were carried out with the streptozotocin-induced diabetes model in Sprague-Dawley rats. Male rats weighing 200 260 grams at study initiation were used. Diabetes was induced by a single intravenous injection of

35 streptozotocin at 50 mg of streptozotocin in sodium citrate buffer per kg of body weight. Non-diabetic

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control rats received a single intravenous injection of
sodium citrate buffer for control purposes. KGF was
administered daily as a subcutaneous injection. The KGF
dose was 3 or 5 mg/kg/day, depending upon the experiment.
5   In the first experiment, KGF therapy was initiated two
days before diabetes, was induced and continued after the
induction of diabetes for a total of eight injections.
In the second and third experiments, KGF therapy
administered subcutaneously was initiated one day after
10   the induction of diabetes with streptozotocin. In the
fourth experiment, a 7 day course of KGF therapy was
initiated 7 days after streptozotocin treatment and the
animals were then followed for an additional 12 weeks.
In all experiments, except for the fourth experiment,
15   blood glucose levels, urine glucose levels and urine
volume were used as end points for analysis.
Additionally, water intake, urine C-peptide levels, or
total pancreatic insulin and C-peptide content were
measured in some experiments. In the fourth experiment,
20   the only assessed endpoint was blood glucose.
Because a large fraction of insulin is removed from the
circulation by the liver, measurement of peripheral
insulin concentrations reflect post-hepatic metabolism
events rather than insulin secretion from the pancreas.
25   Therefore, measurements of C-peptide are often made and
used as a peripheral marker of insulin secretion. C-
peptide is produced from the processing of pro-insulin to
insulin. Insulin and C-peptide are secreted from the
beta cells in equimolar amounts, and only a small amount
30   of C-peptide is extracted by the liver.
      This study investigated the effect of rKGF on
streptozotocin-induced diabetes in Sprague-Dawley rats.
On day 0, groups of rats were exposed to either 45 or 50
mg/kg streptozotocin (STZ). Following these treatments,
35   non-fasting blood glucose levels were monitored daily to
assess the severity of the islet injury. On day 5, the

STZ-treated animals were placed into one of two groups (20/group) depending on the magnitude of hyperglycemia. The dividing point was set at a blood glucose level of 300 mg/dl. A group of non STZ-treated animals served as
5 controls. On day 7, 10 animals from each hyperglycemic group were given ΔN23 (3 mg/kg/day) or PBS by subcutaneous injection for 7 days. Blood glucose levels were then monitored daily, every other day, or weekly and are set forth in Figure 51. Note that STZ-treated
10 animals from both groups receiving ΔN23 had significant declines in blood glucose during the ΔN23 dosing period. Importantly, the mean blood glucose drop experienced by the STZ-treated animals from the <300 mg/dl starting blood glucose group stabilized at about 150 mg/dl whereas
15 the blood glucose drop seen in the >300 mg/dl starting blood glucose group was only transient. Note that the day scale is non-linear.

While the present invention has been described above both generally and in terms of preferred
20 embodiments, it is understood that other variations and modifications will occur to those skilled in the art in light of the description above.

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WHAT IS CLAIMED IS:

1.   A polypeptide analog of a KGF having up to the first 24 N-terminal amino acids modified wherein cysteine residues corresponding to amino acid positions 1 and 15 of the KGF [amino acid positions 32 and 46 of SEQ ID NO:2, (Cys^1 and Cys^{15}, respectively)] are deleted or substituted with another amino acid.

2.   The polypeptide analog of Claim 1 wherein Cys^1 and Cys^{15} are deleted.

3.   The polypeptide analog of Claim 1 wherein the first 15 to 24 N-terminal amino acids are truncated.

4.   The polypeptide analog of Claim 1 wherein Cys^1 is deleted and Cys^{15} is substituted with another amino acid.

5.   The polypeptide analog of Claim 1 wherein up to the first fourteen N-terminal amino acids are truncated and Cys^{15} is substituted with another amino acid.

6.   The polypeptide analog of Claim 1 wherein Cys^1 and Cys^{15} are substituted with another amino acid.

7.   The polypeptide analog as in any one of Claims 4, 5 or 6 wherein an amino acid selected from the group consisting of alanine, leucine, and serine is substituted for cysteine.

8.   The polypeptide analog according to Claim 7 wherein a serine is substituted for a cysteine.

9.   The polypeptide analog according to Claim 1 selected from the group consisting of C(1,15)S, NΔ15, NΔ16, NΔ17, NΔ18, NΔ19, NΔ20, NΔ21, NΔ22, NΔ23 and NΔ24, ΔN3/C(15)S, ΔN3/C(15)-, ΔN8/C(15)S, ΔN8/C(15)-,

5   C(1,15)S/R(144)E, C(1,15)S/R(144)Q and ΔN23/R(144)Q.

10.   A pharmaceutical formulation comprising a therapeutically effective amount of a polypeptide analog according to Claim 1 and a pharmaceutically acceptable

10   carrier.

11.   A pharmaceutical formulation comprising a therapeutically effective amount of a lyophilized polypeptide analog according to Claim 1.

15

12.   The pharmaceutical formulation of Claim 11 further comprising a pharmaceutically acceptable carrier.

20   13.   A nucleic acid molecule selected from the group consisting of DNA and RNA wherein the nucleic acid molecule encodes a polypeptide analog of a KGF having up to the first 24 N-terminal amino acids modified wherein cysteine residues corresponding to amino acid positions

25   1 and 15 of KGF [amino acid positions 32 and 46 of SEQ ID NO:2 (Cys[1] and Cys[15], respectively)] are deleted or substituted with another amino acid.

14.   The nucleic acid molecule of Claim 13 encoding

30   a polypeptide KGF analog wherein Cys[1] and Cys[15] are deleted.

15.   The nucleic acid molecule of Claim 13 encoding a polypeptide KGF analog wherein the first 15-24 N-

35   terminal amino acids are truncated.

16. The nucleic acid molecule of Claim 13 encoding a polypeptide KGF analog wherein Cys^1 is deleted and Cys^{15} is substituted with another amino acid.

17. The nucleic acid molecule of Claim 13 encoding a polypeptide KGF analog wherein up to the first fourteen N-terminal amino acids are truncated and Cys^{15} is substituted with another amino acid.

18. The nucleic acid molecule of Claim 13 encoding a polypeptide KGF analog wherein Cys^1 and Cys^{15} are substituted with another amino acid.

19. The nucleic acid molecule as in any one of Claims 16, 17 or 18 wherein an amino acid selected from the group consisting of alanine, leucine, and serine is substituted for cysteine.

20. A nucleic acid molecule according to Claim 19 wherein a codon coding for serine is substituted for a codon coding for cysteine.

21. The nucleic acid molecule according to Claim 13 wherein the polypeptide analog is selected from the group consisting of C(1,15)S, $N\Delta15$, $N\Delta16$, $N\Delta17$, $N\Delta18$, $N\Delta19$, $N\Delta20$, $N\Delta21$, $N\Delta22$, $N\Delta23$ and $N\Delta24$, $\Delta N3/C(15)S$, $\Delta N3/C(15)-$, $\Delta N8/C(15)S$, $\Delta N8/C(15)-$ and $\Delta N23/R(144)Q$.

22. A biologically functional plasmid or viral vector comprising a nucleic acid molecule according to Claim 13.

23. A procaryotic or eucaryotic host cell stably transfected or transformed with a biologically functional vector according to Claim 22.

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24. A procaryotic host cell according to Claim 23 that is *E. coli*.

5   25. A eucaryotic host cell according to Claim 23 that is a mammalian cell.

26. A eucaryotic host cell according to Claim 25 that is a Chinese hamster ovary cell.

10   27. A process for the production of a polypeptide analog of KGF, the process comprising growing under suitable nutrient conditions a procaryotic or eucaryotic host cell stably transformed with a nucleic acid molecule according to Claim 13, in a manner allowing 15 expression of the encoded polypeptide analog, and isolating the polypeptide analog so produced.

28. A method of stimulating the production of non-fibroblast epithelial cells comprising contacting such 20 cells with an effective amount of a polypeptide analog of a KGF according to Claim 1.

Figure 1

human KGF (+ signal sequence)

```
  |---OLIGO#25-----|                 |-------OLIGO#1---------|
  5'CAATCTACAATTCACAGA 3'        5'CAATGACCTAGGAGTAACAATCAAC 3'
  5'CAATCTACAATTCACAGATAGGAAGAGGTCAATGACCTAGGAGTAACAATCAACTCAAGA-
  ---------+---------+---------+---------+---------+---------+  60


  -TTCATTTTCATTATGTTATTCATGAACACCCGGAGCACTACACTATAATGCACAAATGGA-
  ---------+---------+---------+---------+---------+---------+ 120
                                                      M  H  K  W  I


  -TACTGACATGGATCCTGCCAACTTTGCTCTACAGATCATGCTTTCACATTATCTGTCTAG-
  ---------+---------+---------+---------+---------+---------+ 180
     L  T  W  I  L  P  T  L  L  Y  R  S  C  F  H  I  I  C  L  V


  -TGGGTACTATATCTTTAGCTTGCAATGACATGACTCCAGAGCAAATGGCTACAAATGTGA-
  ---------+---------+---------+---------+---------+---------+ 240
     G  T  I  S  L  A  C  N  D  M  T  P  E  Q  M  A  T  N  V  N


  -ACTGTTCCAGCCCTGAGCGACACACAAGAAGTTATGATTACATGGAAGGAGGGGATATAA-
  ---------+---------+---------+---------+---------+---------+ 300
     C  S  S  P  E  R  H  T  R  S  Y  D  Y  M  E  G  G  D  I·R


  -GAGTGAGAAGACTCTTCTGTCGAACACAGTGGTACCTGAGGATCGATAAAAGAGGCAAAG-
  ---------+---------+---------+---------+---------+---------+ 360
     V  R  R  L  F  C  R  T  Q  W  Y  L  R  I  D  K  R  G  K  V


  -TAAAAGGGACCCAAGAGATGAAGAATAATTACAATATCATGGAAATCAGGACAGTGGCAG-
  ---------+---------+---------+---------+---------+---------+ 420
     K  G  T  Q  E  M  K  N  N  Y  N  I  M  E  I  R  T  V  A  V


  -TTGGAATTGTGGCAATCAAAGGGGTGGAAAGTGAATTCTATCTTGCAATGAACAAGGAAG-
  ---------+---------+---------+---------+---------+---------+ 480
     G  I  V  A  I  K  G  V  E  S  E  F  Y  L  A  M  N  K  E  G


  -GAAAACTCTATGCAAAGAAAGAATGCAATGAAGATTGTAACTTCAAAGAACTAATTCTGG-
  ---------+---------+---------+---------+---------+---------+ 540
     K  L  Y  A  K  K  E  C  N  E  D  C  N  F  K  E  L  I  L  E


  -AAAACCATTACAACACATATGCATCAGCTAAATGGACACACAACGGAGGGGAAATGTTTG-
  ---------+---------+---------+---------+---------+---------+ 600
     N  H  Y  N  T  Y  A  S  A  K  W  T  H  N  G  G  E  M  F  V


  -TTGCCTTAAATCAAAAGGGGGATTCCTGTAAGAGGAAAAAAAACGAAGAAAGAACAAAAAA-
  ---------+---------+---------+---------+---------+---------+ 660
     A  L  N  Q  K  G  I  P  V  R  G  K  K  T  K  K  E  Q  K  T


  -CAGCCCACTTTCTTCCTATGGCAATAACTTAATTGCATATGGTATATAAAGAACCCAGTT
  ---------+---------+---------+---------+---------+---------+ 720
     A  H  F  L  P  M  A  I  T  *
                        3'GGATACCGTTATTGAATT 5'
                         |----OLIGO#26----|
```

Figure 1
(continued)

```
-CCAGCAGGGAGATTTCTTTAAGTGGACTGTTTTCTTTCTTCTCAAAATTTTCTTTCCTTT
 ---------+---------+---------+---------+---------+---------+ 780

-TATTTTTAGTAATCAAGAAAGGCTGGAAAAACTACTGAAAAACTGATCAAGCTGGACTT
 ---------+---------+---------+---------+---------+---------+ 840
                                                     3'ACCTGAA-
                                                     |------

-GTGCATTTATGTTTGTTTTAAG 3'
 ---------+---------+-- 862
-CACGTAAATACAAACAAAA 5'
 ---OLIGO#2--------|
```

Figure 2
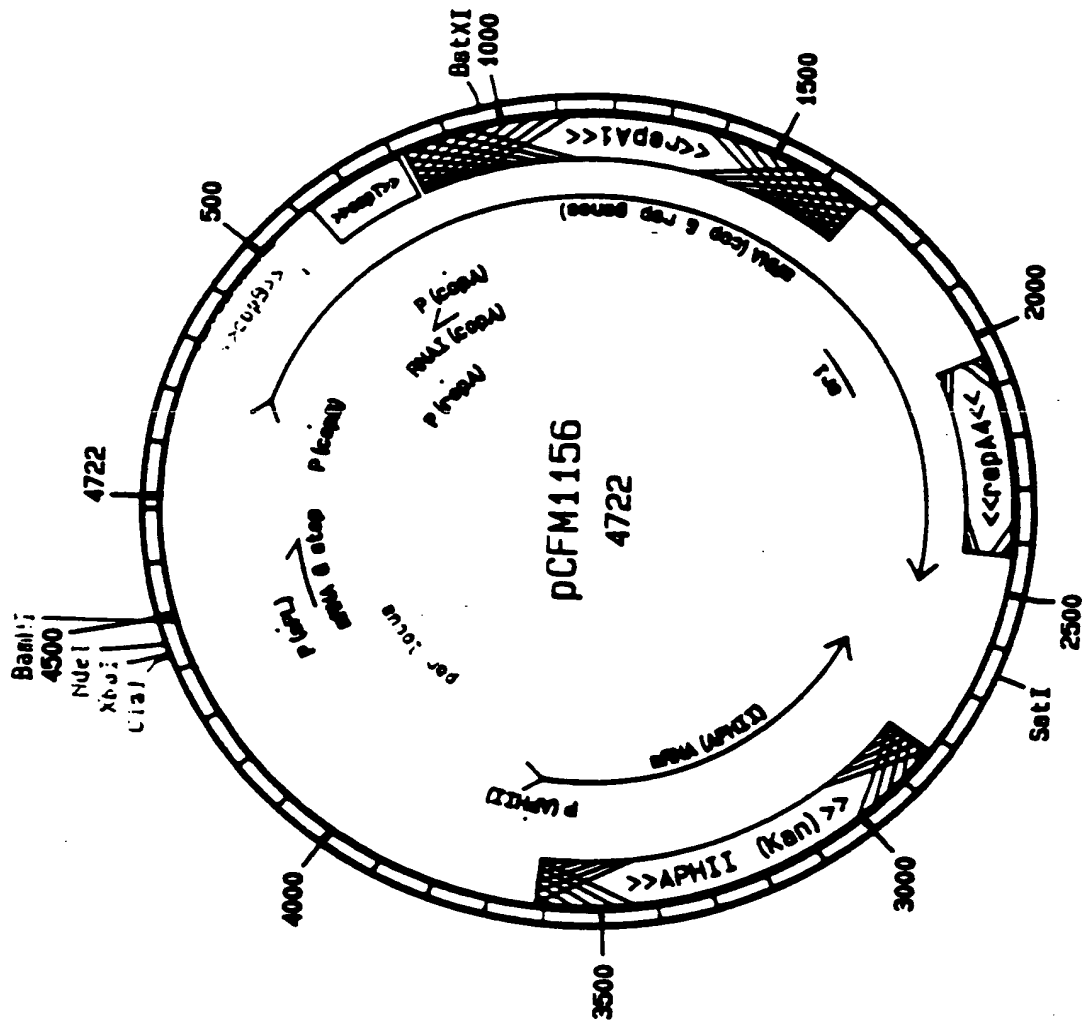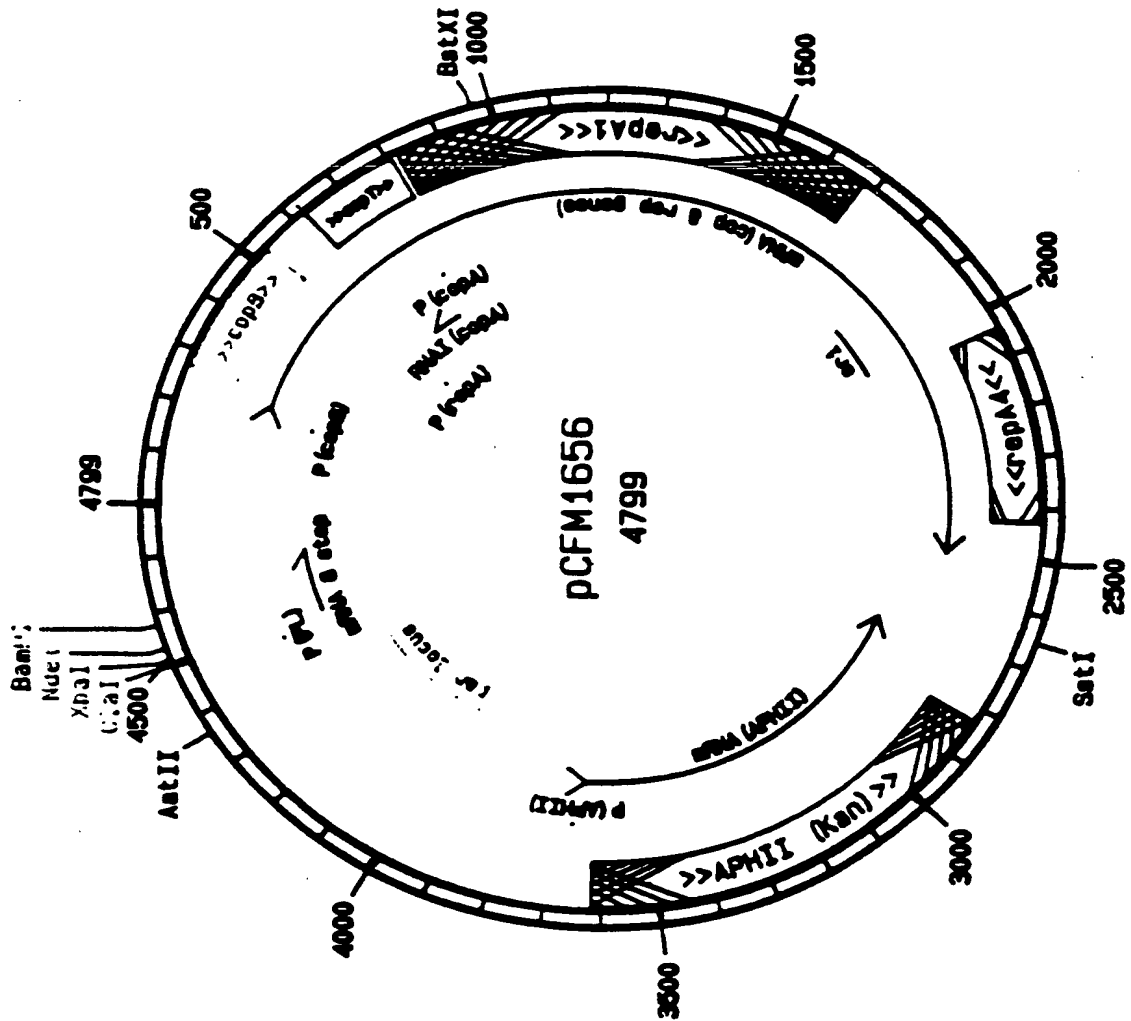
Figure 2
(continued)

Figure 2
(continued)
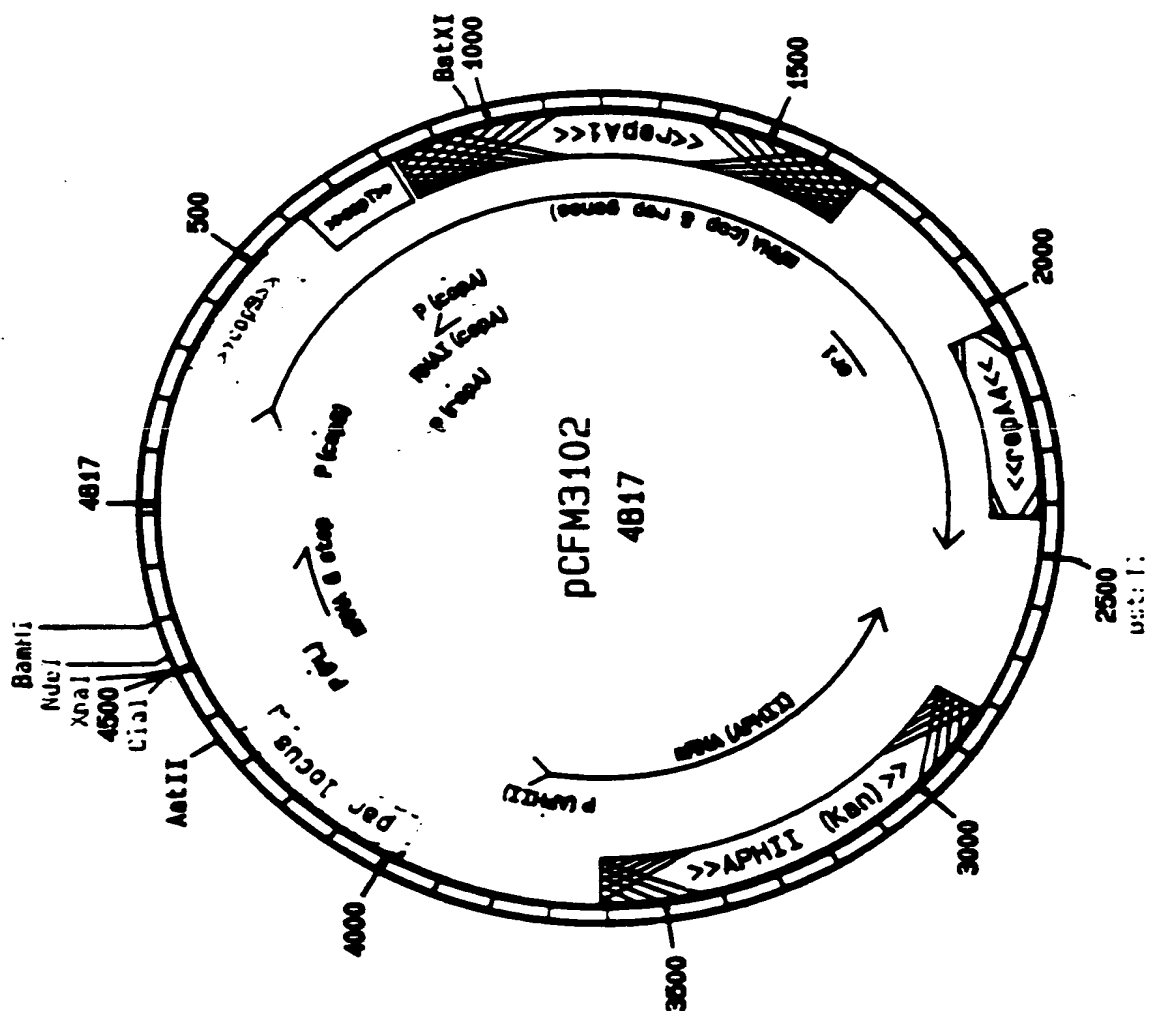
Figure 3

RSH-KGF

```
plasmid DNA          ClaI        XbaI              NdeI
sequence      5'-ATCGATTTGATTCTAGAAGGAGGAATAACATATGAAAAAG-
                                                   M   K   K


             RSH signal sequence                      MluI
-CGCGCACGTGCTATCGCCATTGCTGTGGCTCTGGCAGGTTTCGCAACTAGTGCACA-3'
 R   A   R   A   I   A   I   A   V   A   L   A   G   F   A   T   S   A   H   A   -


MluI
5'CGCGTGCAATGACATGACTCCAGAGCAAATGGCTACAAATGTGAACTGTTCCAGCCCTGA-
  ---------+---------+---------+---------+---------+---------+  60
   -   C   N   D   M   T   P   E   Q   M   A   T   N   V   N   C   S   S   P   E

-GCGACACACAAGAAGTTATGATTACATGGAAGGAGGGGATATAAGAGTGAGAAGACTCTT-
 ---------+---------+---------+---------+---------+---------+ 120
   R   H   T   R   S   Y   D   Y   M   E   G   G   D   I   R   V   R   R   L   F

          KpnI         ClaI
-CTGTCGAACACAGTGGTACCTGAGGATCGATAAAAGAGGCAAAGTAAAAGGGACCCAAGA-
 ---------+---------+---------+---------+---------+---------+ 180
   C   R   T   Q   W   Y   L   R   I   D   K   R   G   K   V   K   G   T   Q   E

-GATGAAGAATAATTACAATATCATGGAAATCAGGACAGTGGCAGTTGGAATTGTGGCAAT-
 ---------+---------+---------+---------+---------+---------+ 240
   M   K   N   N   Y   N   I   M   E   I   R   T   V   A   V   G   I   V   A   I

               EcoRI
-CAAAGGGGTGGAAAGTGAATTCTATCTTGCAATGAACAAGGAAGGAAAACTCTATGCAAA-
 ---------+---------+---------+---------+---------+---------+ 300
   K   G   V   E   S   E   F   Y   L   A   M   N   K   E   G   K   L   Y   A   K

          BsmI
-GAAAGAATGCAATGAAGATTGTAACTTCAAAGAACTAATTCTGGAAAACCATTACAACAC-
 ---------+---------+---------+---------+---------+---------+ 360
   K   E   C   N   E   D   C   N   F   K   E   L   I   L   E   N   H   Y   N   T

NdeI
-ATATGCATCAGCTAAATGGACACACAACGGAGGGGAAATGTTTGTTGCCTTAAATCAAAA-
 ---------+---------+---------+---------+---------+---------+ 420
   Y   A   S   A   K   W   T   H   N   G   G   E   M   F   V   A   L   N   Q   K

-GGGGATTCCTGTAAGAGGAAAAAAAACGAAGAAAGAACAAAAAACAGCCCACTTTCTTCC-
 ---------+---------+---------+---------+---------+---------+ 480
   G   I   P   V   R   G   K   K   T   K   K   E   Q   K   T   A   H   F   L   P

          BamHI
-TATGGCAATAACTTAATAG 3'                   -plasmid DNA
 ---------+---------+--- 503               -sequence
   M   A   I   T   *   *
```

Figure 4

KGF

NdeI
```
5'TATGTGCAATGACATGACTCCAGAGCAAATGGCTACAAATGTGAACTGTTCCAGCCCTGA-
  ---------+---------+---------+---------+---------+---------+ 60
    M  C  N  D  M  T  P  E  Q  M  A  T  N  V  N  C  S  S  P  E

 -GCGACACACAAGAAGTTATGATTACATGGAAGGAGGGGATATAAGAGTGAGAAGACTCTT-
  ---------+---------+---------+---------+---------+---------+ 120
    R  H  T  R  S  Y  D  Y  M  E  G  G  D  I  R  V  R  R  L  F
```

KpnI   ClaI
```
 -CTGTCGAACACAGTGGTACCTGAGGATCGATAAAAGAGGCAAAGTAAAAGGGACCCAAGA-
  ---------+---------+---------+---------+---------+---------+ 180
    C  R  T  Q  W  Y  L  R  I  D  K  R  G  K  V  K  G  T  Q  E

 -GATGAAGAATAATTACAATATCATGGAAATCAGGACAGTGGCAGTTGGAATTGTGGCAAT-
  ---------+---------+---------+---------+---------+---------+ 240
    M  K  N  N  Y  N  I  M  E  I  R  T  V  A  V  G  I  V  A  I
```

EcoRI
```
 -CAAAGGGGTGGAAAGTGAATTCTATCTTGCAATGAACAAGGAAGGAAAACTCTATGCAAA-
  ---------+---------+---------+---------+---------+---------+ 300
    K  G  V  E  S  E  F  Y  L  A  M  N  K  E  G  K  L  Y  A  K
```

BsmI
```
 -GAAAGAATGCAATGAAGATTGTAACTTCAAAGAACTAATTCTGGAAAACCATTACAACAC-
  ---------+---------+---------+---------+---------+---------+ 360
    K  E  C  N  E  D  C  N  F  K  E  L  I  L  E  N  H  Y  N  T
```

NdeI
```
 -ATATGCATCAGCTAAATGGACACACAACGGAGGGGAAATGTTTGTTGCCTTAAATCAAAA-
  ---------+---------+---------+---------+---------+---------+ 420
    Y  A  S  A  K  W  T  H  N  G  G  E  M  F  V  A  L  N  Q  K

 -GGGGATTCCTGTAAGAGGAAAAAAAAACGAAGAAAGAACAAAAAACAGCCCACTTTCTTCC-
  ---------+---------+---------+---------+---------+---------+ 480
    G  I  P  V  R  G  K  K  T  K  K  E  Q  K  T  A  H  F  L  P
```

BamHI
```
 -TATGGCAATAACTTAATAG 3'
  ---------+---------+--- 503
    M  A  I  T  *
```

Figure 5

substitution of KpnI to EcoRI sequence to make KGF(dsd)

```
KpnI
   |-----------OLIGO#6------------------||------OLIGO#7---------
5'    CTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCCAAGAGATGAAAAACAACTACAAT-
3'CATGGACGCATAGCTGTTTGCGCCGTTTCAGTTCCCGTGGGTTCTCTACTTTTTGTTGATGTTA-
   |----------------OLIGO#9-------------------||--OLIGO#10--------
   -  L  R  I  D  K  R  G  K  V  K  G  T  Q  E  M  K  N  N  Y  N  -

                                                              EcoRI
   --------------------------||----------OLIGO#8------------------|
5'-ATTATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCAATCAAAGGTGTTGAATCTG    3'
3'-TAATACCTTTAGGCATGACAACGACAACCATAGCAACGTTAGTTTCCACAACTTAGACTTAA 5'
   -------------------------||----OLIGO#11---------------------|
   I  M  E  I  R  T  V  A  V  G  I  V  A  I  K  G  V  E  S  E  -
```

FIGURE 6

KGF (codon optimized)

```
            XbaI
      |-----OLIGO#12-----|
  5'AGTTTTGATCTAGAAGGAGG 3'
      |-------------------------------OLIGO#14------------------------
  5'AGTTTTGATCTAGAAGGAGGAATAACATATGTGCAACGACATGACTCCGGAACAGATGGCT-

      --------------------------------||--------------OLIGO#15--------
      -ACCAACGTTAACTGCTCCAGCCCGGAACGTCACACCCGTAGCTACGACTACATGGAAGGTG-
                          3' GGGCCTTGCAGTGTGGGCAT 5'
                          |-----OLIGO#20-----|

      --------------------OLIGO#15------------------------------||-
      -GTGACATCCGTGTTCGTCGTCTGTTCTGCCGTACCCAGTGGTACCTGCGTATCGACAAACG-
                                              3' ATAGCTGTTTGC-
                                              |-OLIGO#21--

      ------------------------OLIGO#16-----------------------------
      -TGGTAAAGTTAAAGGTACCCAGGAAATGAAAAACAACTACAACATCATGGAAATCCGTACT-
      -ACCATTTC 5'
      --------|

      -----------------------------||------------OLIGO#17-----------
      -GTTGCTGTTGGTATCGTTGCAATCAAAGGTGTTGAATCTGAATTCTACCTGGCAATGAACA-
                          3' ACGTTAGTTTCCACAACTTA 5'
                          |-----OLIGO#22-----|

      -------------------OLIGO#17------------------------------||----
      -AAGAAGGTAAACTGTACGCAAAAAAAGAATGCAACGAAGACTGCAACTTCAAAGAACTGAT-
                                              3'GAAGTTTCTTGACTA-
                                              |---OLIGO#23---

      ------------------------OLIGO#18-----------------------------
      -CCTGGAAAACCACTACAACACCTACGCATCTGCTAAATGGACCCACAACGGTGGTGAAATG-
      -GGACC 5'
      ----|

      -----------------------||--------------------OLIGO#19---------
      -TTCGTTGCTCTGAACCAGAAAGGTATCCCGGTTCGTGGTAAAAAAACCAAAAAAGAACAGA-
                          3' GGTCTTTCCATAGGGCCAAG 5'
                          |-----OLIGO#24-----|

      -------OLIGO#19-----------------------------------------|
      -AAACCGCTCACTTCCTGCCGATGGCAATCACTTAATAGGATCCAGTTTTGA 3'
                                      3' AATTATCCTAGGTCAAAACT 5'
                                      |----OLIGO#13------|
                                              BamHI
```

Figure 7

KGF C(1,15)S

```
5'ATGTCTAATGATATGACTCCGGAACAGATGGCTACCAACGTTAACTCCTCCTCCCCGGAA-
  ---------+---------+---------+---------+---------+---------+ 60
  M  S  N  D  M  T  P  E  Q  M  A  T  N  V  N  S  S  S  P  E

-CGTCACACGCGTTCCTACGACTACATGGAAGGTGGTGACATCCGCGTACGTCGTCTGTTC-
  ---------+---------+---------+---------+---------+---------+ 120
  R  H  T  R  S  Y  D  Y  M  E  G  G  D  I  R  V  R  R  L  F

-TGCCGTACCCAGTGGTACCTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCCAAGAG-
  ---------+---------+---------+---------+---------+---------+ 180
  C  R  T  Q  W  Y  L  R  I  D  K  R  G  K  V  K  G  T  Q  E

-ATGAAAAACAACTACAATATTATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCAATC-
  ---------+---------+---------+---------+---------+---------+ 240
  M  K  N  N  Y  N  I  M  E  I  R  T  V  A  V  G  I  V  A  I

-AAAGGTGTTGAATCTGAATTCTACCTGGCAATGAACAAAGAAGGTAAACTGTACGCAAAA-
  ---------+---------+---------+---------+---------+---------+ 300
  K  G  V  E  S  E  F  Y  L  A  M  N  K  E  G  K  L  Y  A  K

-AAAGAATGCAACGAAGACTGCAACTTCAAAGAACTGATCCTGGAAAACCACTACAACACC-
  ---------+---------+---------+---------+---------+---------+ 360
  K  E  C  N  E  D  C  N  F  K  E  L  I  L  E  N  H  Y  N  T

-TACGCATCTGCTAAATGGACCCACAACGGTGGTGAAATGTTCGTTGCTCTGAACCAGAAA-
  ---------+---------+---------+---------+---------+---------+ 420
  Y  A  S  A  K  W  T  H  N  G  G  E  M  F  V  A  L  N  Q  K

-GGTATCCCGGTTCGTGGTAAAAAAACCAAAAAAGAACAGAAAACCGCTCACTTCCTGCCG-
  ---------+---------+---------+---------+---------+---------+ 480
  G  I  P  V  R  G  K  K  T  K  K  E  Q  K  T  A  H  F  L  P

-ATGGCAATCACTTAA 3'
  ---------+----- 495
  M  A  I  T  *
```

Figure 8

KGF ΔN3/C(15)S

```
5'ATGACTCCGGAACAGATGGCTACCAACGTTAACTCCTCCTCCCCGGAACGTCACACGCGT-
   ---------+---------+---------+---------+---------+---------+  60
   M  T  P  E  Q  M  A  T  N  V  N  S  S  S  P  E  R  H  T  R

 -TCCTACGACTACATGGAAGGTGGTGACATCCGCGTACGTCGTCTGTTCTGCCGTACCCAG-
   ---------+---------+---------+---------+---------+---------+ 120
   S  Y  D  Y  M  E  G  G  D  I  R  V  R  R  L  F  C  R  T  Q

 -TGGTACCTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCCAAGAGATGAAAAACAAC-
   ---------+---------+---------+---------+---------+---------+ 180
   W  Y  L  R  I  D  K  R  G  K  V  K  G  T  Q  E  M  K  N  N

 -TACAATATTATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCAATCAAAGGTGTTGAA-
   ---------+---------+---------+---------+---------+---------+ 240
   Y  N  I  M  E  I  R  T  V  A  V  G  I  V  A  I  K  G  V  E

 -TCTGAATTCTACCTGGCAATGAACAAAGAAGGTAAACTGTACGCAAAAAAAGAATGCAAC-
   ---------+---------+---------+---------+---------+---------+ 300
   S  E  F  Y  L  A  M  N  K  E  G  K  L  Y  A  K  K  E  C  N·

 -GAAGACTGCAACTTCAAAGAACTGATCCTGGAAAACCACTACAACACCTACGCATCTGCT-
   ---------+---------+---------+---------+---------+---------+ 360
   E  D  C  N  F  K  E  L  I  L  E  N  H  Y  N  T  Y  A  S ·A

 -AAATGGACCCACAACGGTGGTGAAATGTTCGTTGCTCTGAACCAGAAAGGTATCCCGGTT-
   ---------+---------+---------+---------+---------+---------+ 420
   K  W  T  H  N  G  G  E  M  F  V  A  L  N  Q  K  G  I  P  V

 -CGTGGTAAAAAAACCAAAAAAGAACAGAAAACCGCTCACTTCCTGCCGATGGCAATCACT-
   ---------+---------+---------+---------+---------+---------+ 480
   R  G  K  K  T  K  K  E  Q  K  T  A  H  F  L  P  M  A  I  T

 -TAA 3'
 --- 483
 *
```

Figure 9

KGF ΔN3/C(15)-

```
5'ATGACTCCGGAACAGATGGCTACCAACGTTAACTCTTCTCCTGAACGTCATACGCGTTCC-
  ---------+---------+---------+---------+---------+---------+ 60
   M  T  P  E  Q  M  A  T  N  V  N  S  S  P  E  R  H  T  R  S

-TACGACTACATGGAAGGTGGTGACATCCGCGTACGTCGTCTGTTCTGCCGTACCCAGTGG-
  ---------+---------+---------+---------+---------+---------+ 120
   Y  D  Y  M  E  G  G  D  I  R  V  R  R  L  F  C  R  T  Q  W

-TACCTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCCAAGAGATGAAAAACAACTAC-
  ---------+---------+---------+---------+---------+---------+ 180
   Y  L  R  I  D  K  R  G  K  V  K  G  T  Q  E  M  K  N  N  Y

-AATATTATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCAATCAAAGGTGTTGAATCT-
  ---------+---------+---------+---------+---------+---------+ 240
   N  I  M  E  I  R  T  V  A  V  G  I  V  A  I  K  G  V  E  S

-GAATTCTACCTGGCAATGAACAAAGAAGGTAAACTGTACGCAAAAAAAGAATGCAACGAA-
  ---------+---------+---------+---------+---------+---------+ 300
   E  F  Y  L  A  M  N  K  E  G  K  L  Y  A  K  K  E  C  N  E

-GACTGCAACTTCAAAGAACTGATCCTGGAAAACCACTACAACACCTACGCATCTGCTAAA-
  ---------+---------+---------+---------+---------+---------+ 360
   D  C  N  F  K  E  L  I  L  E  N  H  Y  N  T  Y  A  S  A  K

-TGGACCCACAACGGTGGTGAAATGTTCGTTGCTCTGAACCAGAAAGGTATCCCGGTTCGT-
  ---------+---------+---------+---------+---------+---------+ 420
   W  T  H  N  G  G  E  M  F  V  A  L  N  Q  K  G  I  P  V  R

-GGTAAAAAAACCAAAAAAGAACAGAAAACCGCTCACTTCCTGCCGATGGCAATCACTTAA 3'
  ---------+---------+---------+---------+---------+---------+ 480
   G  K  K  T  K  K  E  Q  K  T  A  H  F  L  P  M  A  I  T  *
```

Figure 10

KGF ΔN8/C(15)S

```
5'ATGGCTACTAATGTTAACTCCTCTTCTCCTGAACGTCATACGCGTTCCTACGACTACATG-
  ---------+---------+---------+---------+---------+---------+ 60
   M  A  T  N  V  N  S  S  S  P  E  R  H  T  R  S  Y  D  Y  M

-GAAGGTGGTGACATCCGCGTACGTCGTCTGTTCTGCCGTACCCAGTGGTACCTGCGTATC-
 ---------+---------+---------+---------+---------+---------+ 120
   E  G  G  D  I  R  V  R  R  L  F  C  R  T  Q  W  Y  L  R  I

-GACAAACGCGGCAAAGTCAAGGGCACCCAAGAGATGAAAAACAACTACAATATTATGGAA-
 ---------+---------+---------+---------+---------+---------+ 180
   D  K  R  G  K  V  K  G  T  Q  E  M  K  N  N  Y  N  I  M  E

-ATCCGTACTGTTGCTGTTGGTATCGTTGCAATCAAAGGTGTTGAATCTGAATTCTACCTG-
 ---------+---------+---------+---------+---------+---------+ 240
   I  R  T  V  A  V  G  I  V  A  I  K  G  V  E  S  E  F  Y  L

-GCAATGAACAAAGAAGGTAAACTGTACGCAAAAAAAGAATGCAACGAAGACTGCAACTTC-
 ---------+---------+---------+---------+---------+---------+ 300
   A  M  N  K  E  G  K  L  Y  A  K  K  E  C  N  E  D  C  N  F

-AAAGAACTGATCCTGGAAAACCACTACAACACCTACGCATCTGCTAAATGGACCCACAAC-
 ---------+---------+---------+---------+---------+---------+ 360
   K  E  L  I  L  E  N  H  Y  N  T  Y  A  S  A  K  W  T  H  N

-GGTGGTGAAATGTTCGTTGCTCTGAACCAGAAAGGTATCCCGGTTCGTGGTAAAAAAACC-
 ---------+---------+---------+---------+---------+---------+ 420
   G  G  E  M  F  V  A  L  N  Q  K  G  I  P  V  R  G  K  K  T

-AAAAAAGAACAGAAAACCGCTCACTTCCTGCCGATGGCAATCACTTAA 3'
 ---------+---------+---------+---------+-------- 468
   K  K  E  Q  K  T  A  H  F  L  P  M  A  I  T  *
```

Figure 11

KGF ΔN8/C15-

```
5'ATGGCTACTAATGTTAACTCTTCTCCTGAACGTCATACGCGTTCCTACGACTACATGGAA-
  ---------+---------+---------+---------+---------+---------+ 60
   M  A  T  N  V  N  S  S  P  E  R  H  T  R  S  Y  D  Y  M  E

 -GGTGGTGACATCCGCGTACGTCGTCTGTTCTGCCGTACCCAGTGGTACCTGCGTATCGAC-
  ---------+---------+---------+---------+---------+---------+ 120
   G  G  D  I  R  V  R  R  L  F  C  R  T  Q  W  Y  L  R  I  D

 -AAACGCGGCAAAGTCAAGGGCACCCAAGAGATGAAAAACAACTACAATATTATGGAAATC-
  ---------+---------+---------+---------+---------+---------+ 180
   K  R  G  K  V  K  G  T  Q  E  M  K  N  N  Y  N  I  M  E  I

 -CGTACTGTTGCTGTTGGTATCGTTGCAATCAAAGGTGTTGAATCTGAATTCTACCTGGCA-
  ---------+---------+---------+---------+---------+---------+ 240
   R  T  V  A  V  G  I  V  A  I  K  G  V  E  S  E  F  Y  L  A

 -ATGAACAAAGAAGGTAAACTGTACGCAAAAAAAGAATGCAACGAAGACTGCAACTTCAAA-
  ---------+---------+---------+---------+---------+---------+ 300
   M  N  K  E  G  K  L  Y  A  K  K  E  C  N  E  D  C  N  F  K

 -GAACTGATCCTGGAAAACCACTACAACACCTACGCATCTGCTAAATGGACCCACAACGGT-
  ---------+---------+---------+---------+---------+---------+ 360
   E  L  I  L  E  N  H  Y  N  T  Y  A  S  A  K  W  T  H  N  G

 -GGTGAAATGTTCGTTGCTCTGAACCAGAAAGGTATCCCGGTTCGTGGTAAAAAAACCAAA-
  ---------+---------+---------+---------+---------+---------+ 420
   G  E  M  F  V  A  L  N  Q  K  G  I  P  V  R  G  K  K  T  K

 -AAAGAACAGAAAACCGCTCACTTCCTGCCGATGGCAATCACTTAA 3'
  ---------+---------+---------+---------+----- 465
   K  E  Q  K  T  A  H  F  L  P  M  A  I  T  *
```

Figure 12

KGF ΔN15

```
5'ATGTCTTCTCCTGAACGTCATACGCGTTCCTACGACTACATGGAAGGTGGTGACATCCGC-
  ---------+---------+---------+---------+---------+---------+ 60
   M   S   S   P   E   R   H   T   R   S   Y   D   Y   M   E   G   G   D   I   R

-GTACGTCGTCTGTTCTGCCGTACCCAGTGGTACCTGCGTATCGACAAACGCGGCAAAGTC-
  ---------+---------+---------+---------+---------+---------+ 120
   V   R   R   L   F   C   R   T   Q   W   Y   L   R   I   D   K   R   G   K   V

-AAGGGCACCCAAGAGATGAAAAACAACTACAATATTATGGAAATCCGTACTGTTGCTGTT-
  ---------+---------+---------+---------+---------+---------+ 180
   K   G   T   Q   E   M   K   N   N   Y   N   I   M   E   I   R   T   V   A   V

-GGTATCGTTGCAATCAAAGGTGTTGAATCTGAATTCTACCTGGCAATGAACAAAGAAGGT-
  ---------+---------+---------+---------+---------+---------+ 240
   G   I   V   A   I   K   G   V   E   S   E   F   Y   L   A   M   N   K   E   G

-AAACTGTACGCAAAAAAAGAATGCAACGAAGACTGCAACTTCAAAGAACTGATCCTGGAA-
  ---------+---------+---------+---------+---------+---------+ 300
   K   L   Y   A   K   K   E   C   N   E   D   C   N   F   K   E   L   I   L   E

-AACCACTACAACACCTACGCATCTGCTAAATGGACCCACAACGGTGGTGAAATGTTCGTT-
  ---------+---------+---------+---------+---------+---------+ 360
   N   H   Y   N   T   Y   A   S   A   K   W   T   H   N   G   G   E   M   F   V

-GCTCTGAACCAGAAAGGTATCCCGGTTCGTGGTAAAAAAACCAAAAAAGAACAGAAAACC-
  ---------+---------+---------+---------+---------+---------+ 420
   A   L   N   Q   K   G   I   P   V   R   G   K   K   T   K   K   E   Q   K   T

-GCTCACTTCCTGCCGATGGCAATCACTTAA 3'
  ---------+---------+---------+ 450
   A   H   F   L   P   M   A   I   T   *
```

Figure 13

KGF ΔN16

```
5'ATGTCTCCTGAACGTCATACGCGTTCCTACGACTACATGGAAGGTGGTGACATCCGCGTA-
  ---------+---------+---------+---------+---------+---------+ 60
   M  S  P  E  R  H  T  R  S  Y  D  Y  M  E  G  G  D  I  R  V

-CGTCGTCTGTTCTGCCGTACCCAGTGGTACCTGCGTATCGACAAACGCGGCAAAGTCAAG-
 ---------+---------+---------+---------+---------+---------+ 120
   R  R  L  F  C  R  T  Q  W  Y  L  R  I  D  K  R  G  K  V  K

-GGCACCCAAGAGATGAAAAACAACTACAATATTATGGAAATCCGTACTGTTGCTGTTGGT-
 ---------+---------+---------+---------+---------+---------+ 180
   G  T  Q  E  M  K  N  N  Y  N  I  M  E  I  R  T  V  A  V  G

-ATCGTTGCAATCAAAGGTGTTGAATCTGAATTCTACCTGGCAATGAACAAAGAAGGTAAA-
 ---------+---------+---------+---------+---------+---------+ 240
   I  V  A  I  K  G  V  E  S  E  F  Y  L  A  M  N  K  E  G  K

-CTGTACGCAAAAAAAGAATGCAACGAAGACTGCAACTTCAAAGAACTGATCCTGGAAAAC-
 ---------+---------+---------+---------+---------+---------+ 300
   L  Y  A  K  K  E  C  N  E  D  C  N  F  K  E  L  I  L  E  N

-CACTACAACACCTACGCATCTGCTAAATGGACCCACAACGGTGGTGAAATGTTCGTTGCT-
 ---------+---------+---------+---------+---------+---------+ 360
   H  Y  N  T  Y  A  S  A  K  W  T  H  N  G  G  E  M  F  V  A

-CTGAACCAGAAAGGTATCCCGGTTCGTGGTAAAAAAACCAAAAAAGAACAGAAAACCGCT-
 ---------+---------+---------+---------+---------+---------+ 420
   L  N  Q  K  G  I  P  V  R  G  K  K  T  K  K  E  Q  K  T  A

-CACTTCCTGCCGATGGCAATCACTTAA 3'
 ---------+---------+------- 447
   H  F  L  P  M  A  I  T  *
```

Figure 14

KGF ΔN17

```
5'ATGCCTGAACGTCATACGCGTTCCTACGACTACATGGAAGGTGGTGACATCCGCGTACGT-
  ---------+---------+---------+---------+---------+---------+ 60
   M  P  E  R  H  T  R  S  Y  D  Y  M  E  G  G  D  I  R  V  R

 -CGTCTGTTCTGCCGTACCCAGTGGTACCTGCGTATCGACAAACGCGGCAAAGTCAAGGGC-
  ---------+---------+---------+---------+---------+---------+ 120
   R  L  F  C  R  T  Q  W  Y  L  R  I  D  K  R  G  K  V  K  G

 -ACCCAAGAGATGAAAAACAACTACAATATTATGGAAATCCGTACTGTTGCTGTTGGTATC-
  ---------+---------+---------+---------+---------+---------+ 180
   T  Q  E  M  K  N  N  Y  N  I  M  E  I  R  T  V  A  V  G  I

 -GTTGCAATCAAAGGTGTTGAATCTGAATTCTACCTGGCAATGAACAAAGAAGGTAAACTG-
  ---------+---------+---------+---------+---------+---------+ 240
   V  A  I  K  G  V  E  S  E  F  Y  L  A  M  N  K  E  G  K  L

 -TACGCAAAAAAAGAATGCAACGAAGACTGCAACTTCAAAGAACTGATCCTGGAAAACCAC-
  ---------+---------+---------+---------+---------+---------+ 300
   Y  A  K  K  E  C  N  E  D  C  N  F  K  E  L  I  L  E  N  H

 -TACAACACCTACGCATCTGCTAAATGGACCCACAACGGTGGTGAAATGTTCGTTGCTCTG-
  ---------+---------+---------+---------+---------+---------+ 360
   Y  N  T  Y  A  S  A  K  W  T  H  N  G  G  E  M  F  V  A  L

 -AACCAGAAAGGTATCCCGGTTCGTGGTAAAAAAACCAAAAAAGAACAGAAAACCGCTCAC-
  ---------+---------+---------+---------+---------+---------+ 420
   N  Q  K  G  I  P  V  R  G  K  K  T  K  K  E  Q  K  T  A  H

 -TTCCTGCCGATGGCAATCACTTAA 3'
  ---------+---------+---- 444
   F  L  P  M  A  I  T  *
```

Figure 15

KGF ΔN18

```
5'ATGGAACGTCATACGCGTTCCTACGACTACATGGAAGGTGGTGACATCCGCGTACGTCGT-
  ---------+---------+---------+---------+---------+---------+ 60
   M  E  R  H  T  R  S  Y  D  Y  M  E  G  G  D  I  R  V  R  R

-CTGTTCTGCCGTACCCAGTGGTACCTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACC-
  ---------+---------+---------+---------+---------+---------+ 120
   L  F  C  R  T  Q  W  Y  L  R  I  D  K  R  G  K  V  K  G  T

-CAAGAGATGAAAAACAACTACAATATTATGGAAATCCGTACTGTTGCTGTTGGTATCGTT-
  ---------+---------+---------+---------+---------+---------+ 180
   Q  E  M  K  N  N  Y  N  I  M  E  I  R  T  V  A  V  G  I  V

-GCAATCAAAGGTGTTGAATCTGAATTCTACCTGGCAATGAACAAAGAAGGTAAACTGTAC-
  ---------+---------+---------+---------+---------+---------+ 240
   A  I  K  G  V  E  S  E  F  Y  L  A  M  N  K  E  G  K  L  Y

-GCAAAAAAAGAATGCAACGAAGACTGCAACTTCAAAGAACTGATCCTGGAAAACCACTAC-
  ---------+---------+---------+---------+---------+---------+ 300
   A  K  K  E  C  N  E  D  C  N  F  K  E  L  I  L  E  N  H  Y

-AACACCTACGCATCTGCTAAATGGACCCACAACGGTGGTGAAATGTTCGTTGCTCTGAAC-
  ---------+---------+---------+---------+---------+---------+ 360
   N  T  Y  A  S  A  K  W  T  H  N  G  G  E  M  F  V  A  L  N

-CAGAAAGGTATCCCGGTTCGTGGTAAAAAAACCAAAAAAGAACAGAAAACCGCTCACTTC-
  ---------+---------+---------+---------+---------+---------+ 420
   Q  K  G  I  P  V  R  G  K  K  T  K  K  E  Q  K  T  A  H  F

-CTGCCGATGGCAATCACTTAA 3'
  ---------+---------+- 441
   L  P  M  A  I  T  *
```

Figure 16

KGF ΔN19

```
5'ATGCGTCATACGCGTTCCTACGACTACATGGAAGGTGGTGACATCCGCGTACGTCGTCTG-
  ---------+---------+---------+---------+---------+---------+ 60
   M  R  H  T  R  S  Y  D  Y  M  E  G  G  D  I  R  V  R  R  L

-TTCTGCCGTACCCAGTGGTACCTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCCAA-
 ---------+---------+---------+---------+---------+---------+ 120
   F  C  R  T  Q  W  Y  L  R  I  D  K  R  G  K  V  K  G  T  Q

-GAGATGAAAAACAACTACAATATTATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCA-
 ---------+---------+---------+---------+---------+---------+ 180
   E  M  K  N  N  Y  N  I  M  E  I  R  T  V  A  V  G  I  V  A

-ATCAAAGGTGTTGAATCTGAATTCTACCTGGCAATGAACAAAGAAGGTAAACTGTACGCA-
 ---------+---------+---------+---------+---------+---------+ 240
   I  K  G  V  E  S  E  F  Y  L  A  M  N  K  E  G  K  L  Y  A

-AAAAAAGAATGCAACGAAGACTGCAACTTCAAAGAACTGATCCTGGAAAACCACTACAAC-
 ---------+---------+---------+---------+---------+---------+ 300
   K  K  E  C  N  E  D  C  N  F  K  E  L  I  L  E  N  H  Y  N

-ACCTACGCATCTGCTAAATGGACCCACAACGGTGGTGAAATGTTCGTTGCTCTGAACCAG-
 ---------+---------+---------+---------+---------+---------+ 360
   T  Y  A  S  A  K  W  T  H  N  G  G  E  M  F  V  A  L  N  Q

-AAAGGTATCCCGGTTCGTGGTAAAAAAACCAAAAAAGAACAGAAAACCGCTCACTTCCTG-
 ---------+---------+---------+---------+---------+---------+ 420
   K  G  I  P  V  R  G  K  K  T  K  K  E  Q  K  T  A  H  F  L

-CCGATGGCAATCACTTAA 3'
 ---------+-------- 438
   P  M  A  I  T  *
```

Figure 17

KGF ΔN20

```
5'ATGCATACTCGTTCCTACGACTACATGGAAGGTGGTGACATCCGCGTACGTCGTCTGTTC-
  ---------+---------+---------+---------+---------+---------+ 60
  M  H  T  R  S  Y  D  Y  M  E  G  G  D  I  R  V  R  R  L  F

-TGCCGTACCCAGTGGTACCTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCCAAGAG-
 ---------+---------+---------+---------+---------+---------+ 120
  C  R  T  Q  W  Y  L  R  I  D  K  R  G  K  V  K  G  T  Q  E

-ATGAAAAACAACTACAATATTATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCAATC-
 ---------+---------+---------+---------+---------+---------+ 180
  M  K  N  N  Y  N  I  M  E  I  R  T  V  A  V  G  I  V  A  I

-AAAGGTGTTGAATCTGAATTCTACCTGGCAATGAACAAAGAAGGTAAACTGTACGCAAAA-
 ---------+---------+---------+---------+---------+---------+ 240
  K  G  V  E  S  E  F  Y  L  A  M  N  K  E  G  K  L  Y  A  K

-AAAGAATGCAACGAAGACTGCAACTTCAAAGAACTGATCCTGGAAAACCACTACAACACC-
 ---------+---------+---------+---------+---------+---------+ 300
  K  E  C  N  E  D  C  N  F  K  E  L  I  L  E  N  H  Y  N  T

-TACGCATCTGCTAAATGGACCCACAACGGTGGTGAAATGTTCGTTGCTCTGAACCAGAAA-
 ---------+---------+---------+---------+---------+---------+ 360
  Y  A  S  A  K  W  T  H  N  G  G  E  M  F  V  A  L  N  Q  K

-GGTATCCCGGTTCGTGGTAAAAAAACCAAAAAAGAACAGAAAACCGCTCACTTCCTGCCG-
 ---------+---------+---------+---------+---------+---------+ 420
  G  I  P  V  R  G  K  K  T  K  K  E  Q  K  T  A  H  F  L  P

-ATGGCAATCACTTAA 3'
 ---------+----- 435
  M  A  I  T  *
```

Figure 18

KGF ΔN21

```
5'ATGACTCGTTCCTACGACTACATGGAAGGTGGTGACATCCGCGTACGTCGTCTGTTCTGC-
  ---------+---------+---------+---------+---------+---------+ 60
  M  T  R  S  Y  D  Y  M  E  G  G  D  I  R  V  R  R  L  F  C

-CGTACCCAGTGGTACCTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCCAAGAGATG-
  ---------+---------+---------+---------+---------+---------+ 120
  R  T  Q  W  Y  L  R  I  D  K  R  G  K  V  K  G  T  Q  E  M

-AAAAACAACTACAATATTATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCAATCAAA-
  ---------+---------+---------+---------+---------+---------+ 180
  K  N  N  Y  N  I  M  E  I  R  T  V  A  V  G  I  V  A  I  K

-GGTGTTGAATCTGAATTCTACCTGGCAATGAACAAAGAAGGTAAACTGTACGCAAAAAAA-
  ---------+---------+---------+---------+---------+---------+ 240
  G  V  E  S  E  F  Y  L  A  M  N  K  E  G  K  L  Y  A  K  K

-GAATGCAACGAAGACTGCAACTTCAAAGAACTGATCCTGGAAAACCACTACAACACCTAC-
  ---------+---------+---------+---------+---------+---------+ 300
  E  C  N  E  D  C  N  F  K  E  L  I  L  E  N  H  Y  N  T  Y

-GCATCTGCTAAATGGACCCACAACGGTGGTGAAATGTTCGTTGCTCTGAACCAGAAAGGT-
  ---------+---------+---------+---------+---------+---------+ 360
  A  S  A  K  W  T  H  N  G  G  E  M  F  V  A  L  N  Q  K  G

-ATCCCGGTTCGTGGTAAAAAAACCAAAAAAGAACAGAAAACCGCTCACTTCCTGCCGATG-
  ---------+---------+---------+---------+---------+---------+ 420
  I  P  V  R  G  K  K  T  K  K  E  Q  K  T  A  H  F  L  P  M

-GCAATCACTTAA 3'
  ---------+-- 432
  A  I  T  *
```

Figure 19

KGF ΔN22

```
5'ATGCGTTCCTACGACTACATGGAAGGTGGTGACATCCGCGTACGTCGTCTGTTCTGCCGT-
  ---------+---------+---------+---------+---------+---------+ 60
   M  R  S  Y  D  Y  M  E  G  G  D  I  R  V  R  R  L  F  C  R

-ACCCAGTGGTACCTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCCAAGAGATGAAA-
  ---------+---------+---------+---------+---------+---------+ 120
   T  Q  W  Y  L  R  I  D  K  R  G  K  V  K  G  T  Q  E  M  K

-AACAACTACAATATTATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCAATCAAAGGT-
  ---------+---------+---------+---------+---------+---------+ 180
   N  N  Y  N  I  M  E  I  R  T  V  A  V  G  I  V  A  I  K  G

-GTTGAATCTGAATTCTACCTGGCAATGAACAAAGAAGGTAAACTGTACGCAAAAAAAGAA-
  ---------+---------+---------+---------+---------+---------+ 240
   V  E  S  E  F  Y  L  A  M  N  K  E  G  K  L  Y  A  K  K  E

-TGCAACGAAGACTGCAACTTCAAAGAACTGATCCTGGAAAACCACTACAACACCTACGCA-
  ---------+---------+---------+---------+---------+---------+ 300
   C  N  E  D  C  N  F  K  E  L  I  L  E  N  H  Y  N  T  Y  A

-TCTGCTAAATGGACCCACAACGGTGGTGAAATGTTCGTTGCTCTGAACCAGAAAGGTATC-
  ---------+---------+---------+---------+---------+---------+ 360
   S  A  K  W  T  H  N  G  G  E  M  F  V  A  L  N  Q  K  G  I

-CCGGTTCGTGGTAAAAAAACCAAAAAAGAACAGAAAACCGCTCACTTCCTGCCGATGGCA-
  ---------+---------+---------+---------+---------+---------+ 420
   P  V  R  G  K  K  T  K  K  E  Q  K  T  A  H  F  L  P  M  A

-ATCACTTAA 3'
 --------- 429
   I  T  *
```

Figure 20

KGF ΔN23

```
5'ATGTCCTACGACTACATGGAAGGTGGTGACATCCGCGTACGTCGTCTGTTCTGCCGTACC-
  ---------+---------+---------+---------+---------+---------+ 60
   M  S  Y  D  Y  M  E  G  G  D  I  R  V  R  R  L  F  C  R  T

-CAGTGGTACCTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCCAAGAGATGAAAAAC-
 ---------+---------+---------+---------+---------+---------+ 120
   Q  W  Y  L  R  I  D  K  R  G  K  V  K  G  T  Q  E  M  K  N

-AACTACAATATTATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCAATCAAAGGTGTT-
 ---------+---------+---------+---------+---------+---------+ 180
   N  Y  N  I  M  E  I  R  T  V  A  V  G  I  V  A  I  K  G  V

-GAATCTGAATTCTACCTGGCAATGAACAAAGAAGGTAAACTGTACGCAAAAAAAGAATGC-
 ---------+---------+---------+---------+---------+---------+ 240
   E  S  E  F  Y  L  A  M  N  K  E  G  K  L  Y  A  K  K  E  C

-AACGAAGACTGCAACTTCAAAGAACTGATCCTGGAAAACCACTACAACACCTACGCATCT-
 ---------+---------+---------+---------+---------+---------+ 300
   N  E  D  C  N  F  K  E  L  I  L  E  N  H  Y  N  T  Y  A  S

-GCTAAATGGACCCACAACGGTGGTGAAATGTTCGTTGCTCTGAACCAGAAAGGTATCCCG -
 ---------+---------+---------+---------+---------+---------+ 360
   A  K  W  T  H  N  G  G  E  M  F  V  A  L  N  Q  K  G  I  P

-GTTCGTGGTAAAAAAACCAAAAAAGAACAGAAAACCGCTCACTTCCTGCCGATGGCAATC-
 ---------+---------+---------+---------+---------+---------+ 420
   V  R  G  K  K  T  K  K  E  Q  K  T  A  H  F  L  P  M  A  I

-ACTTAA 3'
 ------ 426
   T  *
```

Figure 21

KGF ΔN24

```
5'ATGTACGACTACATGGAAGGTGGTGACATCCGCGTACGTCGTCTGTTCTGCCGTACCCAG-
  ---------+---------+---------+---------+---------+---------+ 60
   M  Y  D  Y  M  E  G  G  D  I  R  V  R  R  L  F  C  R  T  Q

-TGGTACCTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCCAAGAGATGAAAAACAAC-
 ---------+---------+---------+---------+---------+---------+ 120
   W  Y  L  R  I  D  K  R  G  K  V  K  G  T  Q  E  M  K  N  N

-TACAATATTATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCAATCAAAGGTGTTGAA-
 ---------+---------+---------+---------+---------+---------+ 180
   Y  N  I  M  E  I  R  T  V  A  V  G  I  V  A  I  K  G  V  E

-TCTGAATTCTACCTGGCAATGAACAAAGAAGGTAAACTGTACGCAAAAAAAGAATGCAAC-
 --------+---------+---------+---------+---------+---------+ 240
   S  E  F  Y  L  A  M  N  K  E  G  K  L  Y  A  K  K  E  C  N

-GAAGACTGCAACTTCAAAGAACTGATCCTGGAAAACCACTACAACACCTACGCATCTGCT-
 ---------+---------+---------+---------+---------+---------+ 300
   E  D  C  N  F  K  E  L  I  L  E  N  H  Y  N  T  Y  A  S  A

-AAATGGACCCACAACGGTGGTGAAATGTTCGTTGCTCTGAACCAGAAAGGTATCCCGGTT-
 ---------+---------+---------+---------+---------+---------+ 360
   K  W  T  H  N  G  G  E  M  F  V  A  L  N  Q  K  G  I  P  V

-CGTGGTAAAAAAACCAAAAAAGAACAGAAAACCGCTCACTTCCTGCCGATGGCAATCACT-
 ---------+---------+---------+---------+---------+---------+ 420
   R  G  K  K  T  K  K  E  Q  K  T  A  H  F  L  P  M  A  I  T

-TAA 3'
 --- 423
 *
```

Figure 22

C(1,15)S/R(144)E

```
5'ATGTCTAATGATATGACTCCGGAACAGATGGCTACCAACGTTAACTCCTCCTCCCCGGAA-
   ---------+---------+---------+---------+---------+---------+ 60
   M  S  N  D  M  T  P  E  Q  M  A  T  N  V  N  S  S  S  P  E

-CGTCACACGCGTTCCTACGACTACATGGAAGGTGGTGACATCCGCGTACGTCGTCTGTTC-
   ---------+---------+---------+---------+---------+---------+ 120
   R  H  T  R  S  Y  D  Y  M  E  G  G  D  I  R  V  R  R  L  F

-TGCCGTACCCAGTGGTACCTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCCAAGAG-
   ---------+---------+---------+---------+---------+---------+ 180
   C  R  T  Q  W  Y  L  R  I  D  K  R  G  K  V  K  G  T  Q  E

-ATGAAAAACAACTACAATATTATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCAATC-
   ---------+---------+---------+---------+---------+---------+ 240
   M  K  N  N  Y  N  I  M  E  I  R  T  V  A  V  G  I  V  A  I

-AAAGGTGTTGAATCTGAATTCTATCTTGCAATGAACAAGGAAGGAAAACTCTATGCAAAG-
   ---------+---------+---------+---------+---------+---------+ 300
   K  G  V  E  S  E  F  Y  L  A  M  N  K  E  G  K  L  Y  A  K

-AAAGAATGCAATGAAGATTGTAACTTCAAAGAACTAATTCTGGAAAACCATTACAACACA-
   ---------+---------+---------+---------+---------+---------+ 360
   K  E  C  N  E  D  C  N  F  K  E  L  I  L  E  N  H  Y  N  T

-TATGCATCTGCTAAATGGACCCACAACGGTGGTGAAATGTTCGTTGCTCTGAACCAGAAA-
   ---------+---------+---------+---------+---------+---------+ 420
   Y  A  S  A  K  W  T  H  N  G  G  E  M  F  V  A  L  N  Q  K

-GGTATCCCTGTTGAAGGTAAGAAAACCAAGAAAGAACAGAAAACCGCTCACTTCCTGCCG-
   ---------+---------+---------+---------+---------+---------+ 480
   G  I  P  V  E  G  K  K  T  K  K  E  Q  K  T  A  H  F  L  P

-ATGGCAATCACTTAA 3'
   ---------+----- 495
   M  A  I  T  *
```

Figure 23

KGF C(1,15)S/R(144)Q

```
5'ATGTCTAATGATATGACTCCGGAACAGATGGCTACCAACGTTAACTCCTCCTCCCCGGAA-
 ---------+---------+---------+---------+---------+---------+ 60
   M  S  N  D  M  T  P  E  Q  M  A  T  N  V  N  S  S  S  P  E

-CGTCACACGCGTTCCTACGACTACATGGAAGGTGGTGACATCCGCGTACGTCGTCTGTTC-
 ---------+---------+---------+---------+---------+---------+ 120
   R  H  T  R  S  Y  D  Y  M  E  G  G  D  I  R  V  R  R  L  F

-TGCCGTACCCAGTGGTACCTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCCAAGAG-
 ---------+---------+---------+---------+---------+---------+ 180
   C  R  T  Q  W  Y  L  R  I  D  K  R  G  K  V  K  G  T  Q  E

-ATGAAAAACAACTACAATATTATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCAATC-
 ---------+---------+---------+---------+---------+---------+ 240
   M  K  N  N  Y  N  I  M  E  I  R  T  V  A  V  G  I  V  A  I

-AAAGGTGTTGAATCTGAATTCTATCTTGCAATGAACAAGGAAGGAAAACTCTATGCAAAG-
 ---------+---------+---------+---------+---------+---------+ 300
   K  G  V  E  S  E  F  Y  L  A  M  N  K  E  G  K  L  Y  A  K

-AAAGAATGCAATGAAGATTGTAACTTCAAAGAACTAATTCTGGAAAACCATTACAACACA-
 ---------+---------+---------+---------+---------+---------+ 360
   K  E  C  N  E  D  C  N  F  K  E  L  I  L  E  N  H  Y  N  T

-TATGCATCTGCTAAATGGACCCACAACGGTGGTGAAATGTTCGTTGCTCTGAACCAGAAA-
 ---------+---------+---------+---------+---------+---------+ 420
   Y  A  S  A  K  W  T  H  N  G  G  E  M  F  V  A  L  N  Q  K

-GGTATCCCTGTTCAAGGTAAGAAAACCAAGAAAGAACAGAAAACCGCTCACTTCCTGCCG-
 ---------+---------+---------+---------+---------+---------+ 480
   G  I  P  V  Q  G  K  K  T  K  K  E  Q  K  T  A  H  F  L  P

-ATGGCAATCACTTAA 3'
 ---------+----- 495
   M  A  I  T  *
```

Figure 24

KGF ΔN23/R(144)Q

```
5'ATGTCCTACGACTACATGGAAGGTGGTGACATCCGCGTACGTCGTCTGTTCTGCCGTACC-
  ---------+---------+---------+---------+---------+---------+ 60
  M  S  Y  D  Y  M  E  G  G  D  I  R  V  R  R  L  F  C  R  T

-CAGTGGTACCTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCCAAGAGATGAAAAAC-
 ---------+---------+---------+---------+---------+---------+ 120
 Q  W  Y  L  R  I  D  K  R  G  K  V  K  G  T  Q  E  M  K  N

-AACTACAATATTATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCAATCAAAGGTGTT-
 ---------+---------+---------+---------+---------+---------+ 180
 N  Y  N  I  M  E  I  R  T  V  A  V  G  I  V  A  I  K  G  V

-GAATCTGAATTCTATCTTGCAATGAACAAGGAAGGAAAACTCTATGCAAAGAAAGAATGC-
 ---------+---------+---------+---------+---------+---------+ 240
 E  S  E  F  Y  L  A  M  N  K  E  G  K  L  Y  A  K  K  E  C-

-AATGAAGATTGTAACTTCAAAGAACTAATTCTGGAAAACCATTACAACACATATGCATCT-
 ---------+---------+---------+---------+---------+---------+ 300
 N  E  D  C  N  F  K  E  L  I  L  E  N  H  Y  N  T  Y  A  S

-GCTAAATGGACCCACAACGGTGGTGAAATGTTCGTTGCTCTGAACCAGAAAGGTATCCCT-
 ---------+---------+---------+---------+---------+---------+ 360
 A  K  W  T  H  N  G  G  E  M  F  V  A  L  N  Q  K  G  I  P

-GTTCAAGGTAAGAAAACCAAGAAAGAACAGAAAACCGCTCACTTCCTGCCGATGGCAATC-
 ---------+---------+---------+---------+---------+---------+ 420
 V  Q  G  K  K  T  K  K  E  Q  K  T  A  H  F  L  P  M  A  I

-ACTTAA 3'
 ------ 426
 T  *
```
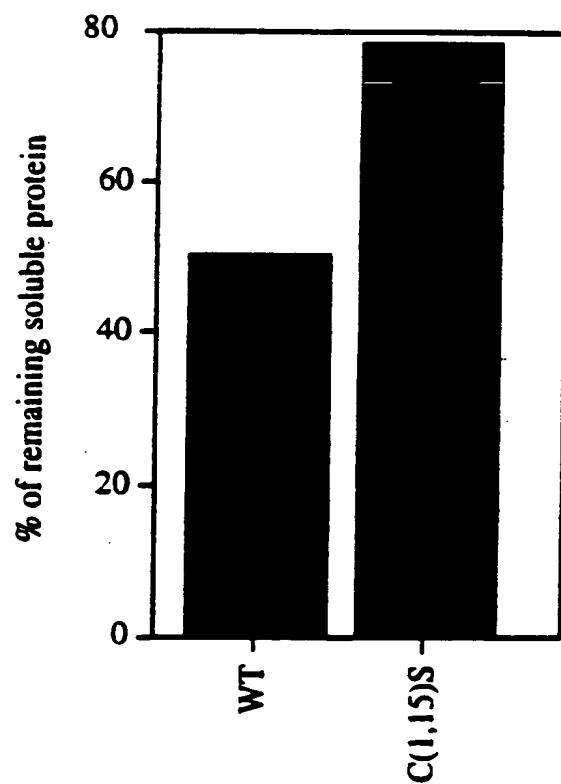
Figure 25

Figure 26
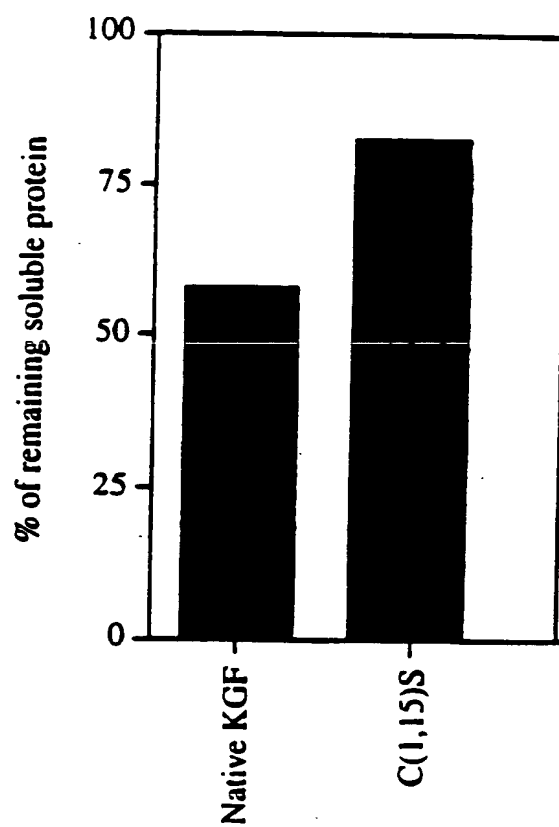
Figure 27
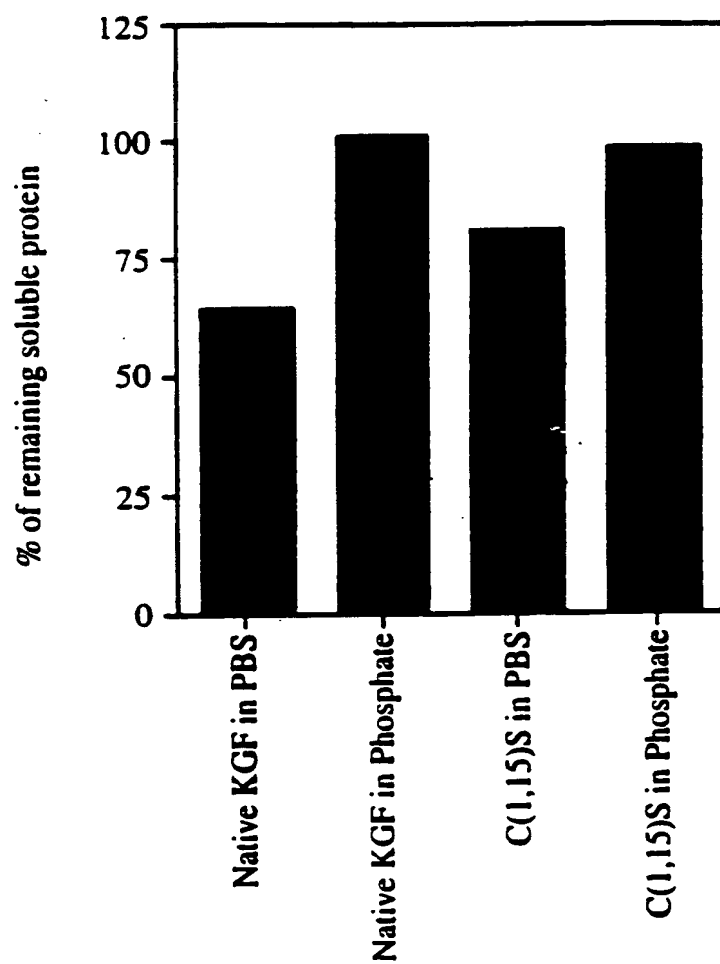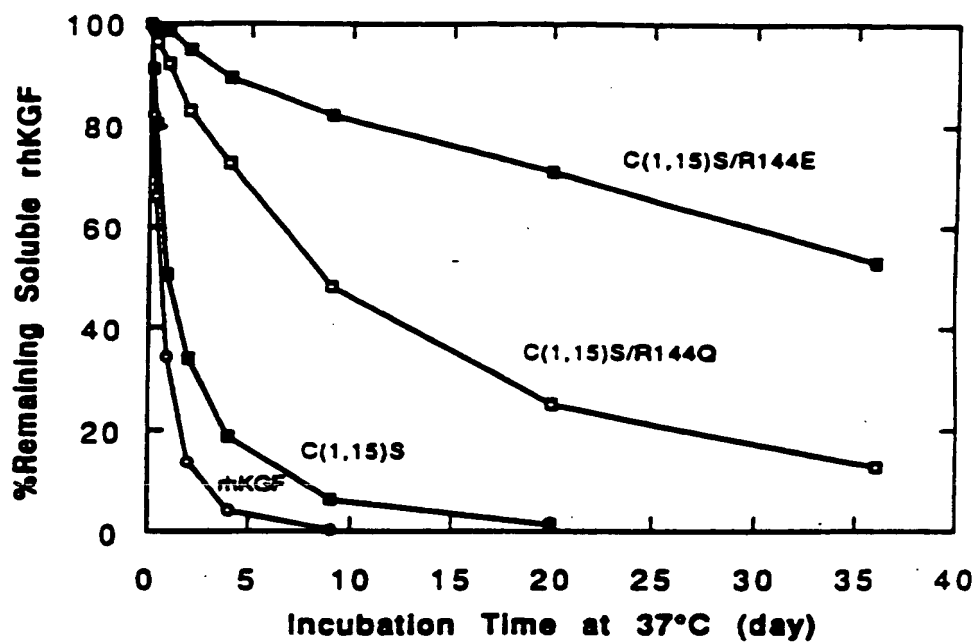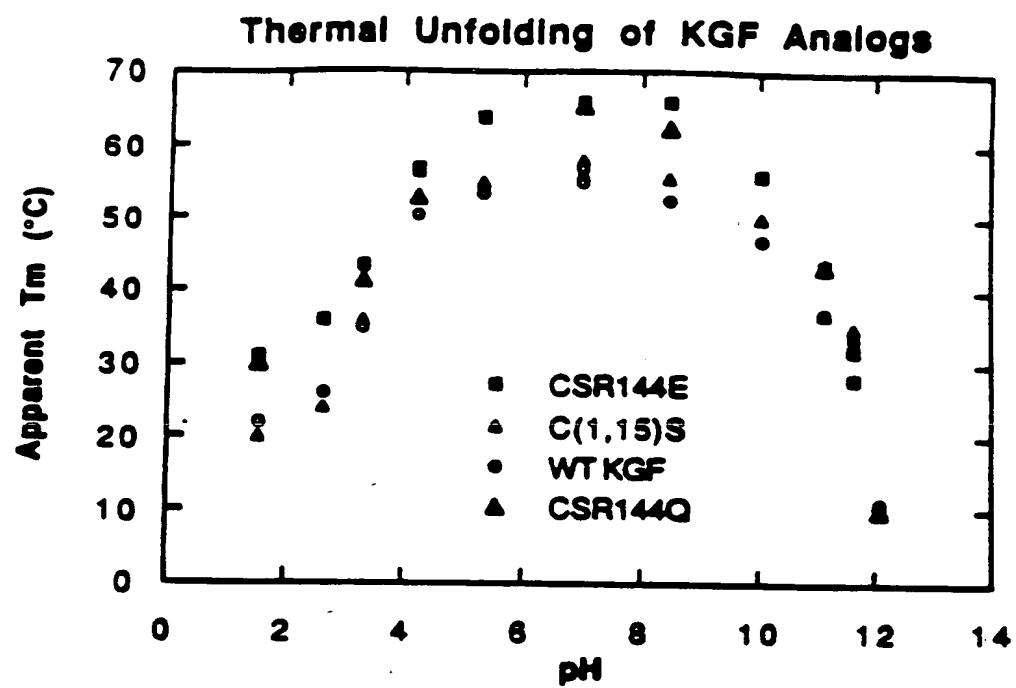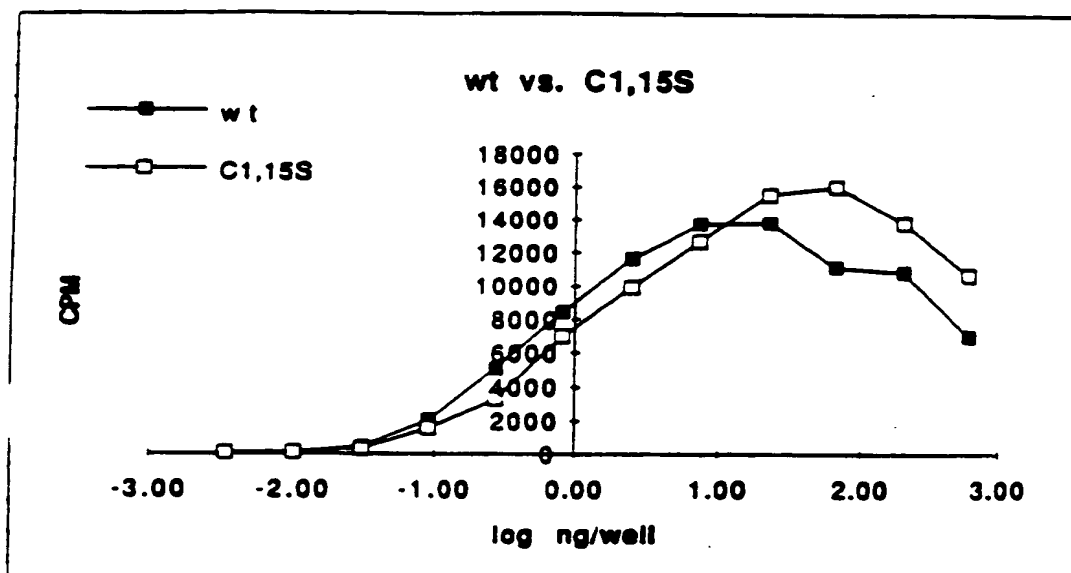
Figure 28

Figure 29

Thermal Unfolding of KGF Analogs

Figure 30

Figure 31

Figure 32
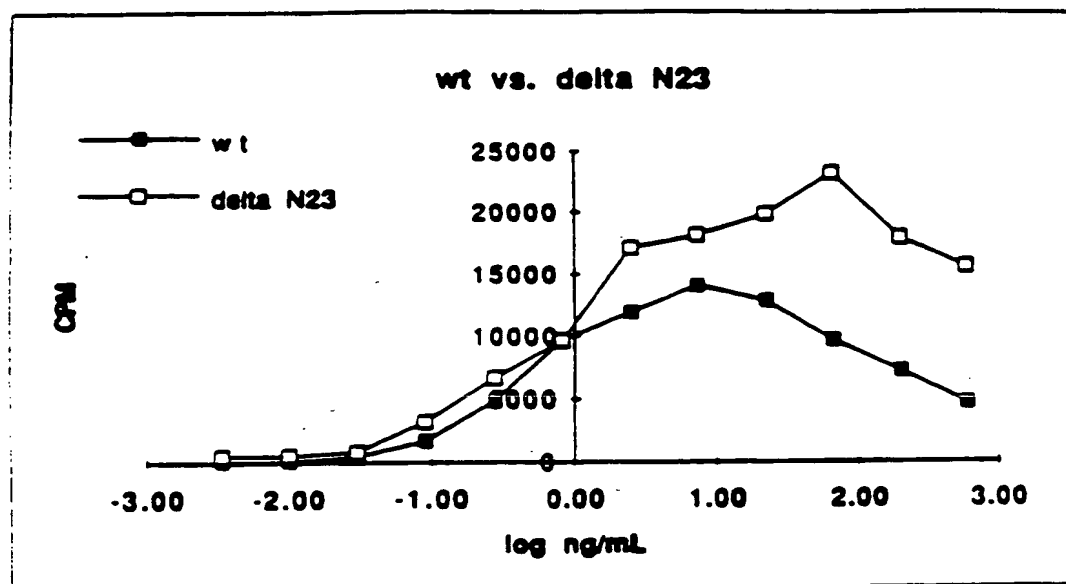
Figure 33
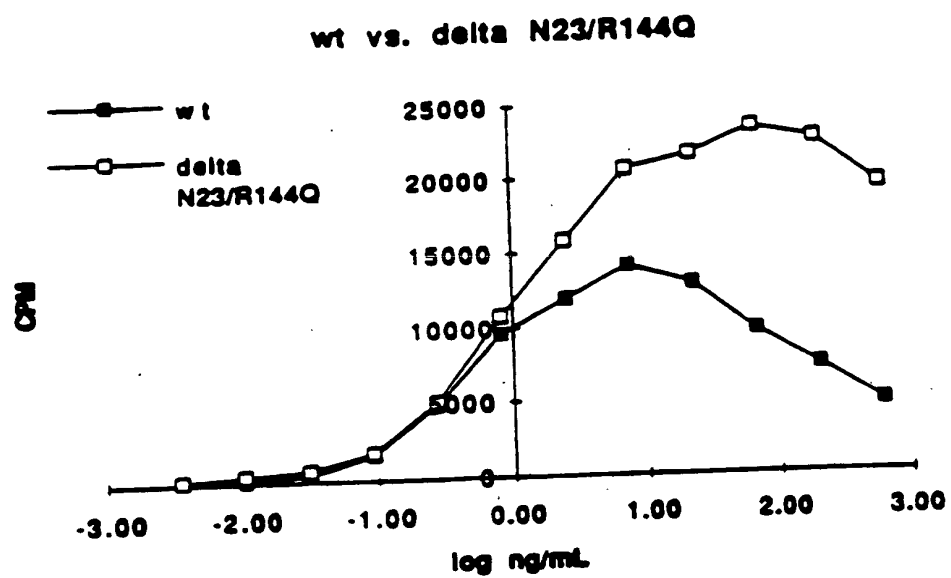

wt vs. delta N23/R144Q
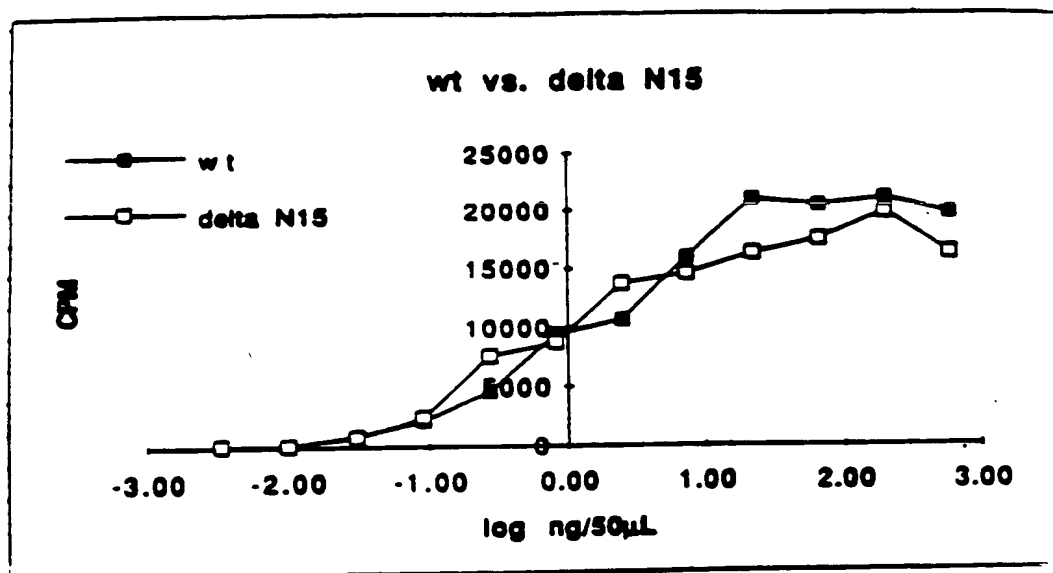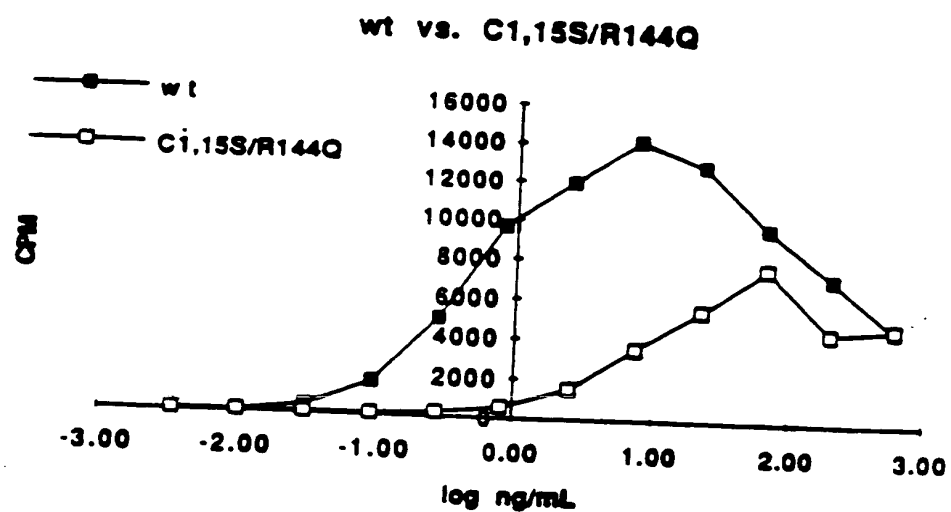
Figure 34

wt vs. C1,15S/R144Q

Figure 35

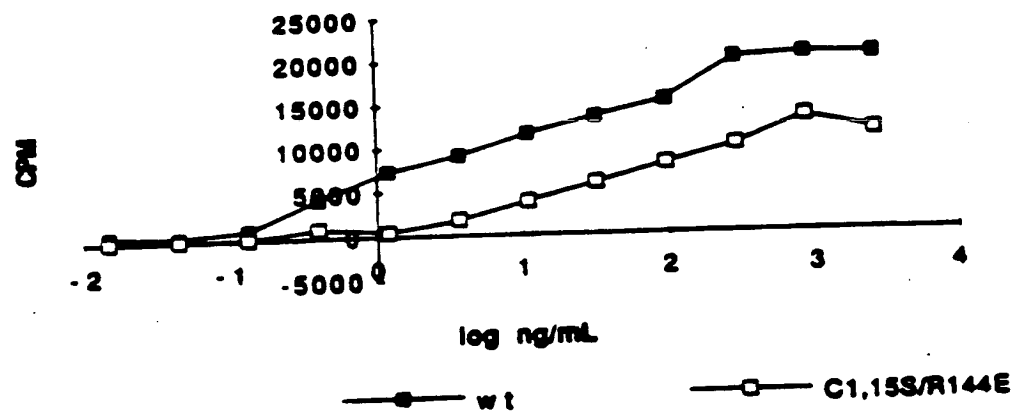wt vs. C1,15S/R144E

Figure 36

Effect of KGF on Serum Cholesterol
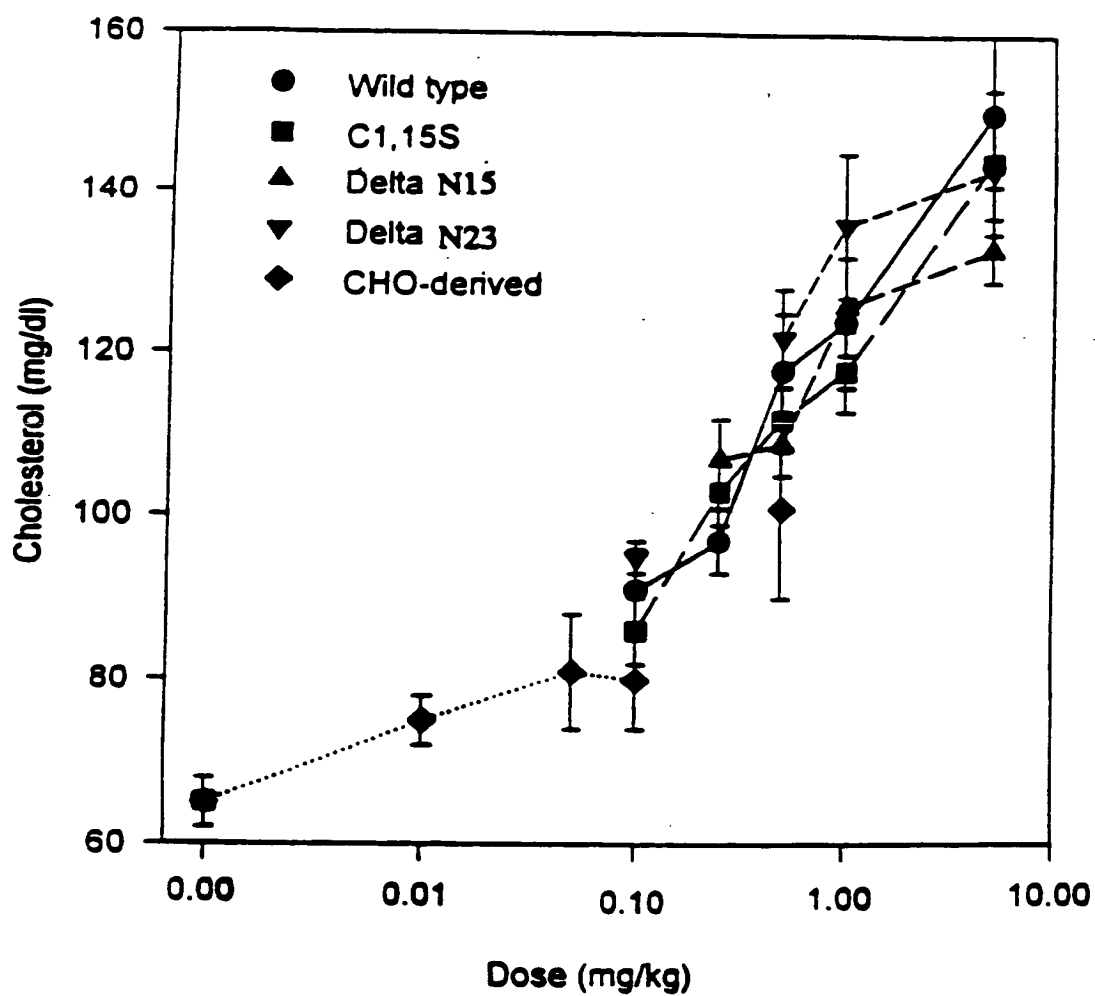
Figure 37

KGF C1,15,40S

```
     CTAGAAGGAGGAATAACATATGTCTAATGATATGACTCCGGAACAGATGGCTACCAACGT
  1  ---------+---------+---------+---------+---------+---------+   60
     TTCCTCCTTATTGTATACAGATTACTATACTGAGGCCTTGTCTACCGATGGTTGCA
                  M  S  N  D  M  T  P  E  Q  M  A  T  N  V

     TAACTCCTCCTCCCCGGAACGTCACACGCGTTCCTACGACTACATGGAAGGTGGTGACAT
 61  ---------+---------+---------+---------+---------+---------+  120
     ATTGAGGAGGAGGGGCCTTGCAGTGTGCGCAAGGATGCTGATGTACCTTCCACCACTGTA
      N  S  S  S  P  E  R  H  T  R  S  Y  D  Y  M  E  G  G  D  I

     CCGCGTACGCCGTTTGTTCTCTCGTACCCAGTGGTACCTGCGTATCGACAAACGCGGCAA
121  ---------+---------+---------+---------+---------+---------+  180
     GGCGCATGCGGCAAACAAGAGAGCATGGGTCACCATGGACGCATAGCTGTTTGCGCCGTT
      R  V  R  R  L  F  S  R  T  Q  W  Y  L  R  I  D  K  R  G  K

     AGTCAAGGGCACCCAAGAGATGAAAAACAACTACAATATTATGGAAATCCGTACTGTTGC
181  ---------+---------+---------+---------+---------+---------+  240
     TCAGTTCCCGTGGGTTCTCTACTTTTTGTTGATGTTATAATACCTTTAGGCATGACAACG
      V  K  G  T  Q  E  M  K  N  N  Y  N  I  M  E  I  R  T  V  A

     TGTTGGTATCGTTGCAATCAAAGGTGTTGAATCTGAATTCTATCTTGCAATGAACAAGGA
241  ---------+---------+---------+---------+---------+---------+  300
     ACAACCATAGCAACGTTAGTTTCCACAACTTAGACTTAAGATAGAACGTTACTTGTTCCT
      V  G  I  V  A  I  K  G  V  E  S  E  F  Y  L  A  M  N  K  E

     AGGAAAACTCTATGCAAAGAAGAATGCAATGAAGATTGTAACTTCAAAGAACTAATTCT
301  ---------+---------+---------+---------+---------+---------+  360
     TCCTTTTGAGATACGTTTCTTTCTTACGTTACTTCTAACATTGAAGTTTCTTGATTAAGA
      G  K  L  Y  A  K  K  E  C  N  E  D  C  N  F  K  E  L  I  L

     GGAAAACCATTACAACACATATGCATCAGCTAAATGGACACACAACGGAGGGGAAATGTT
361  ---------+---------+---------+---------+---------+---------+  420
     CCTTTTGGTAATGTTGTGTATACGTAGTCGATTTACCTGTGTGTTGCCTCCCCTTTACAA
      E  N  H  Y  N  T  Y  A  S  A  K  W  T  H  N  G  G  E  M  F

     TGTTGCCTTAAATCAAAAGGGGATTCCTGTAAGAGGAAAAAAAACGAAGAAAGAACAAAA
421  ---------+---------+---------+---------+---------+---------+  480
     ACAACGGAATTTAGTTTTCCCCTAAGGACATTCTCCTTTTTTTGCTTCTTTCTTGTTTT
      V  A  L  N  Q  K  G  I  P  V  R  G  K  K  T  K  K  E  Q  K

     AACAGCCCACTTTCTTCCTATGGCAATAACTTAATAG
481  ---------+---------+---------+---------+-   521
     TTGTCGGGTGAAAGAAGGATACCGTTATTGAATTATCCTAG
      T  A  H  F  L  P  M  A  I  T  *  *
```

Figure 38

KGF C1,15,102S

```
      CTAGAAGGAGGAATAACATATGTCTAATGATATGACTCCGGAACAGATGGCTACCAACGT
  1   ---------+---------+---------+---------+---------+---------+   60
      TTCCTCCTTATTGTATACAGATTACTATACTGAGGCCTTGTCTACCGATGGTTGCA
                 M  S  N  D  M  T  P  E  Q  M  A  T  N  V

      TAACTCCTCCTCCCCGGAACGTCACACGCGTTCCTACGACTACATGGAAGGTGGTGACAT
 61   ---------+---------+---------+---------+---------+---------+   120
      ATTGAGGAGGAGGGGCCTTGCAGTGTGCGCAAGGATGCTGATGTACCTTCCACCACTGTA
       N  S  S  S  P  E  R  H  T  R  S  Y  D  Y  M  E  G  G  D  I

      CCGCGTACGTCGTCTGTTCTGCCGTACCCAGTGGTACCTGCGTATCGACAAACGCGGCAA
121   ---------+---------+---------+---------+---------+---------+   180
      GGCGCATGCAGCAGACAAGACGGCATGGGTCACCATGGACGCATAGCTGTTTGCGCCGTT
     · R  V  R  R  L  F  C  R  T  Q  W  Y  L  R  I  D  K  R  G  K

      AGTCAAGGGCACCCAAGAGATGAAAAACAACTACAATATTATGGAAATCCGTACTGTTGC
181   ---------+---------+---------+---------+---------+---------+   240
      TCAGTTCCCGTGGGTTCTCTACTTTTTGTTGATGTTATAATACCTTTAGGCATGACAACG
       V  K  G  T  Q  E  M  K  N  N  Y  N  I  M  E  I  R  T  V  A

      TGTTGGTATCGTTGCAATCAAAGGTGTTGAATCTGAATTCTACCTGGCTATGAACAAAGA
241   ---------+---------+---------+---------+---------+---------+   300
      ACAACCATAGCAACGTTAGTTTCCACAACTTAGACTTAAGATGGACCGATACTTGTTTCT
       V  G  I  V  A  I  K  G  V  E  S  E  F  Y  L  A  M  N  K  E

      AGGTAAACTGTACGCTAAAAAGAATCCAATGAAGATTGTAACTTCAAAGAACTAATTCT
301   ---------+---------+---------+---------+---------+---------+   360
      TCCATTTGACATGCGATTTTTTCTTAGGTTACTTCTAACATTGAAGTTTCTTGATTAAGA
       G  K  L  Y  A  K  K  E  S  N  E  D  C  N  F  K  E  L  I  L

      GGAAAACCATTACAACACATATGCATCAGCTAAATGGACACACAACGGAGGGGAAATGTT
361   ---------+---------+---------+---------+---------+---------+   420
      CCTTTTGGTAATGTTGTGTATACGTAGTCGATTTACCTGTGTGTTGCCTCCCCTTTACAA
       E  N  H  Y  N  T  Y  A  S  A  K  W  T  H  N  G  G  E  M  F

      TGTTGCCTTAAATCAAAAGGGGATTCCTGTAAGAGGAAAAAAAACGAAGAAAGAACAAAA
421   ---------+---------+---------+---------+---------+---------+   480
      ACAACGGAATTTAGTTTTCCCCTAAGGACATTCTCCTTTTTTTTGCTTCTTTCTTGTTTT
       V  A  L  N  Q  K  G  I  P  V  R  G  K  K  T  K  K  E  Q  K

      AACAGCCCACTTTCTTCCTATGGCAATAACTTAATAG
481   ---------+---------+---------+--  521
      TTGTCGGGTGAAAGAAGGATACCGTTATTGAATTATCCTAG
       T  A  H  F  L  P  M  A  I  T  *  *
```

Figure 39

KGF C1,15,102,106S

```
     CTAGAAGGAGGAATAACATATGTCTAATGATATGACTCCGGAACAGATGGCTACCAACGT
  1  ---------+---------+---------+---------+---------+---------+   60
     TTCCTCCTTATTGTATACAGATTACTATACTGAGGCCTTGTCTACCGATGGTTGCA
                    M  S  N  D  M  T  P  E  Q  M  A  T  N  V

     TAACTCCTCCTCCCCGGAACGTCACACGCGTTCCTACGACTACATGGAAGGTGGTGACAT
 61  ---------+---------+---------+---------+---------+---------+   120
     ATTGAGGAGGAGGGGCCTTGCAGTGTGCGCAAGGATGCTGATGTACCTTCCACCACTGTA
      N  S  S  S  P  E  R  H  T  R  S  Y  D  Y  M  E  G  G  D  I

     CCGCGTACGTCGTCTGTTCTGCCGTACCCAGTGGTACCTGCGTATCGACAAACGCGGCAA
121  ---------+---------+---------+---------+---------+---------+   180
     GGCGCATGCAGCAGACAAGACGGCATGGGTCACCATGGACGCATAGCTGTTTGCGCCGTT
      R  V  R  R  L  F  C  R  T  Q  W  Y  L  R  I  D  K  R  G  K

     AGTCAAGGGCACCCAAGAGATGAAAAACAACTACAATATTATGGAAATCCGTACTGTTGC
181  ---------+---------+---------+---------+---------+---------+   240
     TCAGTTCCCGTGGGTTCTCTACTTTTTGTTGATGTTATAATACCTTTAGGCATGACAACG
      V  K  G  T  Q  E  M  K  N  N  Y  N  I  M  E  I  R  T  V  A

     TGTTGGTATCGTTGCAATCAAAGGTGTTGAATCTGAATTCTACCTGGCTATGAACAAAGA
241  ---------+---------+---------+---------+---------+---------+   300
     ACAACCATAGCAACGTTAGTTTCCACAACTTAGACTTAAGATGGACCGATACTTGTTTCT
      V  G  I  V  A  I  K  G  V  E  S  E  F  Y  L  A  M  N  K  E

     AGGTAAACTGTACGCTAAAAAAGAATCCAATGAAGATTCTAACTTCAAAGAACTAATTCT
301  ---------+---------+---------+---------+---------+---------+   360
     TCCATTTGACATGCGATTTTTTCTTAGGTTACTTCTAAGATTGAAGTTTCTTGATTAAGA
      G  K  L  Y  A  K  K  E  S  N  E  D  S  N  F  K  E  L  I  L

     GGAAAACCATTACAACACATATGCATCAGCTAAATGGACACACAACGGAGGGGAAATGTT
361  ---------+---------+---------+---------+---------+---------+   420
     CCTTTTGGTAATGTTGTGTATACGTAGTCGATTTACCTGTGTGTTGCCTCCCCTTTACAA
      E  N  H  Y  N  T  Y  A  S  A  K  W  T  H  N  G  G  E  M  F

     TGTTGCCTTAAATCAAAAGGGGATTCCTGTAAGAGGAAAAAAAACGAAGAAAGAACAAAA
421  ---------+---------+---------+---------+---------+---------+   480
     ACAACGGAATTTAGTTTTCCCCTAAGGACATTCTCCTTTTTTTTGCTTCTTTCTTGTTTT
      V  A  L  N  Q  K  G  I  P  V  R  G  K  K  T  K  K  E  Q  K

     AACAGCCCACTTTCTTCCTATGGCAATAACTTAATAG
481  ---------+---------+---------+---------+-  521
     TTGTCGGGTGAAAGAAGGATACCGTTATTGAATTATCCTAG
      T  A  H  F  L  P  M  A  I  T  *  *
```

Figure 40

ΔN23/N(137)E

```
5'-ATGTCCTACGACTACATGGAAGGTGGTGACATCCGCGTACGTCGTCTGTTCTGCCGTACC-
    +---------+---------+---------+---------+---------+---------
     M  S  Y  D  Y  M  E  G  G  D  I  R  V  R  R  L  F  C  R  T  -

  -CAGTGGTACCTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCCAAGAGATGAAAAAC-
    +---------+---------+---------+---------+---------+---------
     Q  W  Y  L  R  I  D  K  R  G  K  V  K  G  T  Q  E  M  K  N  -

  -AACTACAATATTATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCAATCAAAGGTGTT-
    +---------+---------+---------+---------+---------+---------
     N  Y  N  I  M  E  I  R  T  V  A  V  G  I  V  A  I  K  G  V  -

  -GAATCTGAATTCTACCTGGCAATGAACAAAGAAGGTAAACTGTACGCAAAAAAAGAATGC-
    +---------+---------+---------+---------+---------+---------
     E  S  E  F  Y  L  A  M  N  K  E  G  K  L  Y  A  K  K  E  C  -

  -AACGAAGACTGCAACTTCAAAGAACTGATCCTGGAAAACCACTACAACACCTACGCATCT-
    +---------+---------+---------+---------+---------+---------
     N  E  D  C  N  F  K  E  L  I  L  E  N  H  Y  N  T  Y  A  S  -

  -GCTAAATGGACCCACAACGGTGGTGAAATGTTCGTTGCTCTGGAACAGAAAGGTATCCCT-
    +---------+---------+---------+---------+---------+---------
     A  K  W  T  H  N  G  G  E  M  F  V  A  L  E  Q  K  G  I  P  -

  -GTTCGTGGTAAGAAAACCAAGAAAGAACAGAAAACCGCTCACTTCCTGCCGATGGCAATC-
    +---------+---------+---------+---------+---------+---------
     V  R  G  K  K  T  K  K  E  Q  K  T  A  H  F  L  P  M  A  I  -

  -ACTTAA-3'
    +-----
     T  *
```

Figure 41

ΔN23/K(139)E

```
5'-ATGTCCTACGACTACATGGAAGGTGGTGACATCCGCGTACGTCGTCTGTTCTGCCGTACC-
  +---------+---------+---------+---------+---------+---------
   M  S  Y  D  Y  M  E  G  G  D  I  R  V  R  R  L  F  C  R  T  -

-CAGTGGTACCTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCCAAGAGATGAAAAAC
  +---------+---------+---------+---------+---------+---------
   Q  W  Y  L  R  I  D  K  R  G  K  V  K  G  T  Q  E  M  K  N  -

-AACTACAATATTATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCAATCAAAGGTGTT-
  +---------+---------+---------+---------+---------+---------
   N  Y  N  I  M  E  I  R  T  V  A  V  G  I  V  A  I  K  G  V  -

-GAATCTGAATTCTACCTGGCAATGAACAAAGAAGGTAAACTGTACGCAAAAAAAGAATGC-
  +---------+---------+---------+---------+---------+---------
   E  S  E  F  Y  L  A  M  N  K  E  G  K  L  Y  A  K  K  E  C  -

-AACGAAGACTGCAACTTCAAAGAACTGATCCTGGAAAACCACTACAACACCTACGCATCT
  +---------+---------+---------+---------+---------+---------
   N  E  D  C  N  F  K  E  L  I  L  E  N  H  Y  N  T  Y  A  S  -

-GCTAAATGGACCCACAACGGTGGTGAAATGTTCGTTGCTCTGAACCAGGAAGGTATCCCT-
  +---------+---------+---------+---------+---------+---------
   A  K  W  T  H  N  G  G  E  M  F  V  A  L  N  Q  E  G  I  P  -

-GTTCGTGGTAAGAAAACCAAGAAAGAACAGAAAACCGCTCACTTCCTGCCGATGGCAATC-
  +---------+---------+---------+---------+---------+---------
   V  R  G  K  K  T  K  K  E  Q  K  T  A  H  F  L  P  M  A  I  -

-ACTTAA-3'
  +-----
   T  *
```

Figure 42

ΔN23/K(139)Q

```
5'-ATGTCCTACGACTACATGGAAGGTGGTGACATCCGCGTACGTCGTCTGTTCTGCCGTACC-
   +---------+---------+---------+---------+---------+---------+---------
     M  S  Y  D  Y  M  E  G  G  D  I  R  V  R  R  L  F  C  R  T  -

  -CAGTGGTACCTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCCAAGAGATGAAAAAC-
   +---------+---------+---------+---------+---------+---------+---------
     Q  W  Y  L  R  I  D  K  R  G  K  V  K  G  T  Q  E  M  K  N  -

  -AACTACAATATTATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCAATCAAAGGTGTT-
   +---------+---------+---------+---------+---------+---------+---------
     N  Y  N  I  M  E  I  R  T  V  A  V  G  I  V  A  I  K  G  V  -

  -GAATCTGAATTCTACCTGGCAATGAACAAAGAAGGTAAACTGTACGCAAAAAAAGAATGC-
   +---------+---------+---------+---------+---------+---------+---------
     E  S  E  F  Y  L  A  M  N  K  E  G  K  L  Y  A  K  K  E  C  -

  -AACGAAGACTGCAACTTCAAAGAACTGATCCTGGAAAACCACTACAACACCTACGCATCT-
   +---------+---------+---------+---------+---------+---------+---------
     N  E  D  C  N  F  K  E  L  I  L  E  N  H  Y  N  T  Y  A  S  -

  -GCTAAATGGACCCACAACGGTGGTGAAATGTTCGTTGCTCTGAACCAGCAAGGTATCCCT-
   +---------+---------+---------+---------+---------+---------+---------
     A  K  W  T  H  N  G  G  E  M  F  V  A  L  N  Q  Q  G  I  P  -

  -GTTCGTGGTAAGAAAACCAAGAAAGAACAGAAAACCGCTCACTTCCTGCCGATGGCAATC-
   +---------+---------+---------+---------+---------+---------+---------
     V  R  G  K  K  T  K  K  E  Q  K  T  A  H  F  L  P  M  A  I  -

  -ACTTAA-3'
   +-----
     T  *
```

Figure 43

ΔN23/R(144)A

```
5'-ATGTCCTACGACTACATGGAAGGTGGTGACATCCGCGTACGTCGTCTGTTCTGCCGTACC-
   +---------+---------+---------+---------+---------+---------
    M  S  Y  D  Y  M  E  G  G  D  I  R  V  R  R  L  F  C  R  T  -

  -CAGTGGTACCTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCCAAGAGATGAAAAAC-
   +---------+---------+---------+---------+---------+---------
    Q  W  Y  L  R  I  D  K  R  G  K  V  K  G  T  Q  E  M  K  N  -

  -AACTACAATATTATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCAATCAAAGGTGTT-
   +---------+---------+---------+---------+---------+---------
    N  Y  N  I  M  E  I  R  T  V  A  V  G  I  V  A  I  K  G  V  -

  -GAATCTGAATTCTACCTGGCAATGAACAAAGAAGGTAAACTGTACGCAAAAAAAGAATGC-
   +---------+---------+---------+---------+---------+---------
    E  S  E  F  Y  L  A  M  N  K  E  G  K  L  Y  A  K  K  E  C  -

  -AACGAAGACTGCAACTTCAAAGAACTGATCCTGGAAAACCACTACAACACCTACGCATCT-
   +---------+---------+---------+---------+---------+---------
    N  E  D  C  N  F  K  E  L  I  L  E  N  H  Y  N  T  Y  A  S  -

  -GCTAAATGGACCCACAACGGTGGTGAAATGTTCGTTGCTCTGAACCAGAAAGGTATCCCT-
   +---------+---------+---------+---------+---------+---------
    A  K  W  T  H  N  G  G  E  M  F  V  A  L  N  Q  K  G  I  P  -

  -GTTGCTGGTAAGAAAACCAAGAAAGAACAGAAAACCGCTCACTTCCTGCCGATGGCAATC-
   +---------+---------+---------+---------+---------+---------
    V  A  G  K  K  T  K  K  E  Q  K  T  A  H  F  L  P  M  A  I  -

  -ACTTAA-3'
   +-----
    T  *
```

Figure 44

ΔN23/R(144)E

```
5'-ATGTCCTACGACTACATGGAAGGTGGTGACATCCGCGTACGTCGTCTGTTCTGCCGTACC-
   +---------+---------+---------+---------+---------+---------
   M  S  Y  D  Y  M  E  G  G  D  I  R  V  R  R  L  F  C  R  T  -

-CAGTGGTACCTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCCAAGAGATGAAAAAC-
 +---------+---------+---------+---------+---------+---------
 Q  W  Y  L  R  I  D  K  R  G  K  V  K  G  T  Q  E  M  K  N  -

-AACTACAATATTATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCAATCAAAGGTGTT-
 +---------+---------+---------+---------+---------+---------
 N  Y  N  I  M  E  I  R  T  V  A  V  G  I  V  A  I  K  G  V  -

-GAATCTGAATTCTATCTTGCAATGAACAAGGAAGGAAAACTCTATGCAAAGAAAGAATGC-
 +---------+---------+---------+---------+---------+---------
 E  S  E  F  Y  L  A  M  N  K  E  G  K  L  Y  A  K  K  E  C  -

-AATGAAGATTGTAACTTCAAAGAACTAATTCTGGAAAACCATTACAACACATATGCATCT-
 +---------+---------+---------+---------+---------+---------
 N  E  D  C  N  F  K  E  L  I  L  E  N  H  Y  N  T  Y  A  S  -

-GCTAAATGGACCCACAACGGTGGTGAAATGTTCGTTGCTCTGAACCAGAAAGGTATCCCT-
 +---------+---------+---------+---------+---------+---------
 A  K  W  T  H  N  G  G  E  M  F  V  A  L  N  Q  K  G  I  P  -

-GTTGAAGGTAAGAAAACCAAGAAAGAACAGAAAACCGCTCACTTCCTGCCGATGGCAATC-
 +---------+---------+---------+---------+---------+---------
 V  E  G  K  K  T  K  K  E  Q  K  T  A  H  F  L  P  M  A  I  -

-ACTTAA-3'
 +-----
 T  *
```

Figure 45

ΔN23/R(144)L

```
5'-ATGTCCTACGACTACATGGAAGGTGGTGACATCCGCGTACGTCGTCTGTTCTGCCGTACC-
   +---------+---------+---------+---------+---------+---------
    M  S  Y  D  Y  M  E  G  G  D  I  R  V  R  R  L  F  C  R  T  -

-CAGTGGTACCTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCCAAGAGATGAAAAAC-
 +---------+---------+---------+---------+---------+---------
  Q  W  Y  L  R  I  D  K  R  G  K  V  K  G  T  Q  E  M  K  N  -

-AACTACAATATTATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCAATCAAAGGTGTT-
 +---------+---------+---------+---------+---------+---------
  N  Y  N  I  M  E  I  R  T  V  A  V  G  I  V  A  I  K  G  V  -

-GAATCTGAATTCTACCTGGCAATGAACAAAGAAGGTAAACTGTACGCAAAAAAAGAATGC-
 +---------+---------+---------+---------+---------+---------
  E  S  E  F  Y  L  A  M  N  K  E  G  K  L  Y  A  K  K  E  C  -

-AACGAAGACTGCAACTTCAAAGAACTGATCCTGGAAAACCACTACAACACCTACGCATCT-
 +---------+---------+---------+---------+---------+---------
  N  E  D  C  N  F  K  E  L  I  L  E  N  H  Y  N  T  Y  A  S  -

-GCTAAATGGACCCACAACGGTGGTGAAATGTTCGTTGCTCTGAACCAGAAAGGTATCCCT-
 +---------+---------+---------+---------+---------+---------
  A  K  W  T  H  N  G  G  E  M  F  V  A  L  N  Q  K  G  I  P  -

-GTTCTGGGTAAGAAAACCAAGAAAGAACAGAAAACCGCTCACTTCCTGCCGATGGCAATC-
 +---------+---------+---------+---------+---------+---------
  V  L  G  K  K  T  K  K  E  Q  K  T  A  H  F  L  P  M  A  I  -

-ACTTAA-3'
 +-----
  T  *
```

Figure 46

ΔN23/K(147)E

```
5'-ATGTCCTACGACTACATGGAAGGTGGTGACATCCGCGTACGTCGTCTGTTCTGCCGTACC-
   +---------+---------+---------+---------+---------+---------
    M  S  Y  D  Y  M  E  G  G  D  I  R  V  R  R  L  F  C  R  T  -

  -CAGTGGTACCTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCCAAGAGATGAAAAAC-
   +---------+---------+---------+---------+---------+---------
    Q  W  Y  L  R  I  D  K  R  G  K  V  K  G  T  Q  E  M  K  N  -

  -AACTACAATATTATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCAATCAAAGGTGTT-
   +---------+---------+---------+---------+---------+---------
    N  Y  N  I  M  E  I  R  T  V  A  V  G  I  V  A  I  K  G  V  -

  -GAATCTGAATTCTACCTGGCAATGAACAAAGAAGGTAAACTGTACGCAAAAAAAGAATGC-
   +---------+---------+---------+---------+---------+---------
    E  S  E  F  Y  L  A  M  N  K  E  G  K  L  Y  A  K  K  E  C  -

  -AACGAAGACTGCAACTTCAAAGAACTGATCCTGGAAAACCACTACAACACCTACGCATCT-
   +---------+---------+---------+---------+---------+---------
    N  E  D  C  N  F  K  E  L  I  L  E  N  H  Y  N  T  Y  A  S  -

  -GCTAAATGGACCCACAACGGTGGTGAAATGTTCGTTGCTCTGAACCAGAAAGGTATTCCT-
   +---------+---------+---------+---------+---------+---------
    A  K  W  T  H  N  G  G  E  M  F  V  A  L  N  Q  K  G  I  P  -

  -GTTCGTGGTAAGGAAACCAAGAAAGAACAGAAAACCGCTCACTTCCTGCCGATGGCAATC-
   +---------+---------+---------+---------+---------+---------
    V  R  G  K  E  T  K  K  E  Q  K  T  A  H  F  L  P  M  A  I  -

  -ACTTAA-3'
   +-----
    T  *
```

Figure 47

ΔN23/K(147)Q

```
5'-ATGTCCTACGACTACATGGAAGGTGGTGACATCCGCGTACGTCGTCTGTTCTGCCGTACC-
   +---------+---------+---------+---------+---------+---------
    M  S  Y  D  Y  M  E  G  G  D  I  R  V  R  R  L  F  C  R  T  -

  -CAGTGGTACCTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCCAAGAGATGAAAAAC-
   +---------+---------+---------+---------+---------+---------
    Q  W  Y  L  R  I  D  K  R  G  K  V  K  G  T  Q  E  M  K  N  -

  -AACTACAATATTATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCAATCAAAGGTGTT-
   +---------+---------+---------+---------+---------+---------
    N  Y  N  I  M  E  I  R  T  V  A  V  G  I  V  A  I  K  G  V  -

  -GAATCTGAATTCTACCTGGCAATGAACAAAGAAGGTAAACTGTACGCAAAAAAAGAATGC-
   +---------+---------+---------+---------+---------+---------
    E  S  E  F  Y  L  A  M  N  K  E  G  K  L  Y  A  K  K  E  C  -

  -AACGAAGACTGCAACTTCAAAGAACTGATCCTGGAAAACCACTACAACACCTACGCATCT-
   +---------+---------+---------+---------+---------+---------
    N  E  D  C  N  F  K  E  L  I  L  E  N  H  Y  N  T  Y  A  S  -

  -GCTAAATGGACCCACAACGGTGGTGAAATGTTCGTTGCTCTGAACCAGAAAGGTATCCCT-
   +---------+---------+---------+---------+---------+---------
    A  K  W  T  H  N  G  G  E  M  F  V  A  L  N  Q  K  G  I  P  -

  -GTTCGTGGTAAGCAAACCAAGAAAGAACAGAAAACCGCTCACTTCCTGCCGATGGCAATC-
   +---------+---------+---------+---------+---------+---------
    V  R  G  K  Q  T  K  K  E  Q  K  T  A  H  F  L  P  M  A  I  -

  -ACTTAA-3'
   +-----
    T  *
```

Figure 48

ΔN23/K(153)E

```
5'-ATGTCCTACGACTACATGGAAGGTGGTGACATCCGCGTACGTCGTCTGTTCTGCCGTACC-
   +---------+---------+---------+---------+---------+---------+
   M  S  Y  D  Y  M  E  G  G  D  I  R  V  R  R  L  F  C  R  T  -

  -CAGTGGTACCTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCCAAGAGATGAAAAAC-
   +---------+---------+---------+---------+---------+---------+
   Q  W  Y  L  R  I  D  K  R  G  K  V  K  G  T  Q  E  M  K  N  -

  -AACTACAATATTATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCAATCAAAGGTGTT-
   +---------+---------+---------+---------+---------+---------+
   N  Y  N  I  M  E  I  R  T  V  A  V  G  I  V  A  I  K  G  V  -

  -GAATCTGAATTCTACCTGGCAATGAACAAAGAAGGTAAACTGTACGCAAAAAAAGAATGC-
   +---------+---------+---------+---------+---------+---------+
   E  S  E  F  Y  L  A  M  N  K  E  G  K  L  Y  A  K  K  E  C  -

  -AACGAAGACTGCAACTTCAAAGAACTGATCCTGGAAAACCACTACAACACCTACGCATCT-
   +---------+---------+---------+---------+---------+---------+
   N  E  D  C  N  F  K  E  L  I  L  E  N  H  Y  N  T  Y  A  S  -

  -GCTAAATGGACCCACAACGGTGGTGAAATGTTCGTTGCTCTGAACCAGAAAGGTATCCCT-
   +---------+---------+---------+---------+---------+---------+
   A  K  W  T  H  N  G  G  E  M  F  V  A  L  N  Q  K  G  I  P  -

  -GTTCGTGGTAAGAAAACCAAGAAAGAACAGGAAACCGCTCACTTCCTGCCGATGGCAATC-
   +---------+---------+---------+---------+---------+---------+
   V  R  G  K  K  T  K  K  E  Q  E  T  A  H  F  L  P  M  A  I  -

  -ACTTAA-3'
   +-----
   T  *
```

Figure 49

ΔN23/K(153)Q

```
5'-ATGTCCTACGACTACATGGAAGGTGGTGACATCCGCGTACGTCGTCTGTTCTGCCGTACC-
   +---------+---------+---------+---------+---------+---------
   M  S  Y  D  Y  M  E  G  G  D  I  R  V  R  R  L  F  C  R  T  -

- CAGTGGTACCTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCCAAGAGATGAAAAAC-
  +---------+---------+---------+---------+---------+---------
  Q  W  Y  L  R  I  D  K  R  G  K  V  K  G  T  Q  E  M  K  N  -

-AACTACAATATTATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCAATCAAAGGTGTT-
 +---------+---------+---------+---------+---------+---------
 N  Y  N  I  M  E  I  R  T  V  A  V  G  I  V  A  I  K  G  V  -

-GAATCTGAATTCTACCTGGCAATGAACAAAGAAGGTAAACTGTACGCAAAAAAAGAATGC-
 +---------+---------+---------+---------+---------+---------
 E  S  E  F  Y  L  A  M  N  K  E  G  K  L  Y  A  K  K  E  C  -

-AACGAAGACTGCAACTTCAAAGAACTGATCCTGGAAAACCACTACAACACCTACGCATCT-
 +---------+---------+---------+---------+---------+---------
 N  E  D  C  N  F  K  E  L  I  L  E  N  H  Y  N  T  Y  A  S  -

-GCTAAATGGACCCACAACGGTGGTGAAATGTTCGTTGCTCTGAACCAGAAAGGTATCCCT-
 +---------+---------+---------+---------+---------+---------
 A  K  W  T  H  N  G  G  E  M  F  V  A  L  N  Q  K  G  I  P  -

-GTTCGTGGTAAGAAAACCAAGAAAGAACAGCAAACCGCTCACTTCCTGCCGATGGCAATC-
 +---------+---------+---------+---------+---------+---------
 V  R  G  K  K  T  K  K  E  Q  Q  T  A  H  F  L  P  M  A  I  -

-ACTTAA-3'
 +-----
 T  *  -
```

Figure 50

ΔN23/Q(152)E/K(153)E

```
5'-ATGTCCTACGACTACATGGAAGGTGGTGACATCCGCGTACGTCGTCTGTTCTGCCGTACC-
   +---------+---------+---------+---------+---------+---------
    M  S  Y  D  Y  M  E  G  G  D  I  R  V  R  R  L  F  C  R  T  -

  -CAGTGGTACCTGCGTATCGACAAACGCGGCAAAGTCAAGGGCACCCAAGAGATGAAAAAC-
   +---------+---------+---------+---------+---------+---------
    Q  W  Y  L  R  I  D  K  R  G  K  V  K  G  T  Q  E  M  K  N  -

  -AACTACAATATTATGGAAATCCGTACTGTTGCTGTTGGTATCGTTGCAATCAAAGGTGTT-
   +---------+---------+---------+---------+---------+---------
    N  Y  N  I  M  E  I  R  T  V  A  V  G  I  V  A  I  K  G  V  -

  -GAATCTGAATTCTACCTGGCAATGAACAAAGAAGGTAAACTGTACGCAAAAAAAGAATGC-
   +---------+---------+---------+---------+---------+---------
    E  S  E  F  Y  L  A  M  N  K  E  G  K  L  Y  A  K  K  E  C  -

  -AACGAAGACTGCAACTTCAAAGAACTGATCCTGGAAAACCACTACAACACCTACGCATCT-
   +---------+---------+---------+---------+---------+---------
    N  E  D  C  N  F  K  E  L  I  L  E  N  H  Y  N  T  Y  A  S  -

  -GCTAAATGGACCCACAACGGTGGTGAAATGTTCGTTGCTCTGAACCAGAAAGGTATCCCT-
   +---------+---------+---------+---------+---------+---------
    A  K  W  T  H  N  G  G  E  M  F  V  A  L  N  Q  K  G  I  P  -

  -GTTCGTGGTAAGAAAACCAAGAAAGAAGAGGAAACCGCTCACTTCCTGCCGATGGCAATC-
   +---------+---------+---------+---------+---------+---------
    V  R  G  K  K  T  K  K  E  E  E  T  A  H  F  L  P  M  A  I  -

  -ACTTAA-3'
   +-----
    T  *
```

FIGURE 51